Universidade de Aveiro Departamento de Biologia
Ano 2017

Marta Pena Gil Fraga

PAH levels in parturient and newborns from Aveiro region, Portugal

Níveis de HAPs em parturientes e recém-nascidos da região de Aveiro, Portugal

DECLARAÇÃO

Declaro que este relatório é integralmente da minha autoria, estando devidamente referenciadas as fontes e obras consultadas, bem como identificadas de modo claro as citações dessas obras. Não contém, por isso, qualquer tipo de plágio quer de textos publicados, qualquer que seja o meio dessa publicação, incluindo meios eletrónicos, quer de trabalhos académicos.

Universidade de Aveiro Departamento de Biologia
Ano 2017

Marta Pena Gil Fraga

PAH levels in parturient and newborns from Aveiro region, Portugal

Níveis de HAPs em parturientes e recém-nascidos da região de Aveiro, Portugal

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Toxicologia e Ecotoxicologia, realizada sob a orientação científica da Doutora Susana Patrícia Mendes Loureiro, Professora Auxiliar com Agregação do Departamento de Biologia e CESAM (Centro de Estudos do Ambiente e do Mar) da Universidade de Aveiro e co-orientação do Doutor Carlos Alexandre Sarabando Gravato, Professor Auxiliar da Faculdade de Ciências da Universidade de Lisboa e da Doutora Marta Sofia Soares Craveiro Alves Monteiro dos Santos, Investigadora em Pós-Doutoramento do Departamento de Biologia e CESAM da Universidade de Aveiro.

Ao meu avô Fernando que me ajudou a crescer tanto a nível pessoal como intelectual. Obrigada por todos os desafios que me propuseste e que me ajudaste a ultrapassar. Ao meu irmão Miguel por ser a melhor pessoa que surgiu na minha vida. Obrigada.

o júri

Presidente	Professor Doutor Fernando José Mendes Gonçalves Professor Associado com Agregação do Departamento de Biologia da Universidade de Aveiro
Arguente	Professora Doutora Virgília Sofia Almeida de Azevedo e Silva Professora Auxiliar Convidada, Departamento de Biologia, Universidade de Aveiro; Investigadora em Pós-Doutoramento, CESAM, Universidade de Aveiro.
Orientador	Professora Doutora Susana Patrícia Mendes Loureiro Professora Auxiliar com Agregação do Departamento de Biologia e CESAM (Centro de Estudos do Ambiente e do Mar) da Universidade de Aveiro

agradecimentos

Agradeço à minha orientadora Professora Susana Loureiro por me ter facultado este trabalho e todos os ensinamentos. Obrigada por toda a disciplina, espírito crítico e sabedoria que me proporcionou. Sem dúvida, seria impossível ter escolhido melhor uma orientadora para mim.
Agradeço ao meu co-orientador Professor Carlos Gravato por todos os ensinamentos, boa disposição e disponibilidade.
Agradeço à minha co-orientadora Professora Marta Monteiro por me ter apoiado sempre, pela sua disponibilidade e por me ter impulsionado a ser melhor. Penso que sem si não teria conseguido. Obrigada por me ter recebido no seu gabinete sempre que eu estava preocupada e por vezes um bocado perdida. Penso que será impossível colocar por palavras o que me ajudou. Obrigada.
Agradeço ao Professor Amadeus Soares por me ter disponibilizado a oportunidade de usufruir dos equipamentos das suas instalações. Sem elas este trabalho seria impossível.
Ao Abel Ferreira por todo o apoio logístico e técnico.
Obrigada aos meus colegas do ApplEE por todo o apoio. Em especial ao Carlos Silva por me ter ajudado a dar os primeiros passos no laboratório; à Luísa pela ajuda preciosa com a estatística da minha tese; e à Filipa Nogueira pela amizade.
Obrigada Ana Alves pelas amostras. Ambiciono um dia contribuir para a ciência como tu o fizeste.
A todos os meus amigos da Universidade de Aveiro, Niedja Santos, Inês Simões e Beatriz Matos por toda a amizade sem vocês este caminho seria mais difícil de percorrer.
Às minhas colegas de casa, a Ana Velhinho por ser sempre tão prestável e bem-disposta e à Beatriz Cruz que me apoiou desde o início deste mestrado como me conhecesse desde sempre. Às duas: obrigada por estarem sempre presentes.
Aos meus colegas da UTAD em especial à Isabel Rodrigues e ao Tiago Monteiro que apesar de estarem longe estão sempre no meu coração e a um telefonema de distância.
À minha avó Maria Pena, por todo o carinho e reconforto. Ao meu querido Pai e à minha querida Mãe. Obrigada por me apoiarem e me permitirem ter o que tenho hoje. Sem vocês não era nada.

palavras-chave

Hidrocarbonetos aromáticos policíclicos , exposição pré-natal, biomonitorização, biomarcadores de exposição, sangue, placenta

resumo

A exposição humana a hidrocarbonetos aromáticos policíclicos (HAPs) pode advir de fogos florestais, combustão proveniente do carvão para fazer alcatrão, fumo do tabaco e fumo proveniente de tráfego rodoviário, podendo torna-se crítica em algumas áreas de residência ou tipos de trabalho. Os HAPs são considerados um grupo de químicos prioritários uma vez que são carcinogénicos, mutagénicos e teratogénicos. Portanto, é crucial monitorizar e estudar a exposição de populações humanas a HAPs principalmente durante o desenvolvimento fetal, que é considerada uma fase maís sensível à exposição a contaminantes. Neste contexto os principais objetivos deste estudo foram: (1) avaliar a exposição de HAPs nas parturientes e recém-nascidos da região de Aveiro usando como matrizes biológicas sangue e placenta; (2) examinar a influência ambiental, dados sociodemográficos, estilo de vida e hábitos tabágicos que podem contribuir para a exposição dos HAPs durante a gravidez na região de Aveiro, Portugal.

Neste estudo participaram 49 mães e recém-nascidos da região de Aveiro. A amostragem efetuada (placenta, sangue do cordão umbilical e sangue das parturientes) e informações recolhidas foram aprovadas pelo Comité de Ética do Hospital Infante D. Pedro do Centro Hospitalar Baixo Vouga, Aveiro e todas as parturientes assinaram previamente consentimento informado. Foi utilizada espectrofotometria de fluorescência para efetuar a quantificação dos HAPs (homólogos de naftaleno, fenantreno, pireno e benzo[a]pireno) nos tecidos recolhidos.

Em geral o grupo estudado apresentou elevados níveis de HAPs na placenta (fração total homogeneizada) e os níveis mais baixos no sangue do cordão umbilical. Os HAPs de baixo peso molecular (homólogos de naftaleno e fenantreno) que foram medidos na placenta, apresentaram níveis mais elevados que os níveis de HAPs de elevado peso molecular (homólogos de pireno e benzo[a]pireno) encontrados na placenta. Considerando o local de residência, os níveis de HAPs mais elevados na placenta foram encontrados em parturientes que habitavam em Aveiro, Ílhavo e Albergaria-a-Velha e os níveis mais baixos em Águeda. Além disso, o aumento dos níveis de homólogos de naftaleno e fenantreno na placenta foram associados à exposição a fumo proveniente de tráfego rodoviário e os elevados níveis de benzo[a]pireno foram associados com a exposição a tabaco no trabalho. Os níveis mais elevados de homólogos de naftaleno, fenantreno e benzo[a]pireno foram encontrados na placenta (fracção homogeneizada total) de parturientes que fumaram no terceiro trimestre de gravidez. Não foram porém encontradas correlações significativas entre os níveis de HAPs e os dados antropométricos dos recém-nascidos, mas foram em geral encontradas elevadas concentrações de HAPs em recém-nascidos com menor peso, perímetro cefálico e comprimento.

Este projeto permitiu obter uma visão geral dos níveis de HAPs em parturientes do distrito de Aveiro, identificando as principais fontes de exposição a HAPs. Para além disso, verificou-se que a placenta acumula HAPs, mas não funciona como uma barreira total a estes compostos, uma vez que estes conseguem alcançar o sangue do cordão umbilical, podendo como tal vir a causar efeitos adversos nos recém-nascidos.

keywords

Polycyclic aromatic hydrocarbons, pre-natal exposure, biomonitoring, biomarkers of exposure, blood, placenta

abstract

Human exposure to polycyclic aromatic hydrocarbons (PAHs) can arise from forest fires, coal tar combustion, vehicle exhausts and tobacco smoke and can be critical in some residential and working areas. PAHs are considered a group of priority chemicals as they are carcinogenic, mutagenic and teratogenic. Therefore, is crucial to monitor and study human exposure to PAHs, namely during fetal development, which is considered a sensitive window of exposure to contaminants. In this context, the main objectives of this study were: (i) to assess maternal and fetal exposure to PAHs in parturient from the Aveiro region using samples of placenta and blood as biological matrices; (ii) to examine the influence of environmental, sociodemographic, lifestyle and smoking habits that may contribute to PAHs exposure during pregnancy in Aveiro region, Portugal.

This study was performed in 49 mother/newborn pairs from Aveiro region. All the matrices sampled (placenta, umbilical cord blood and mothers' blood) and information gathered was approved by Ethics Committee of Infante D. Pedro Hospital in Centro Hospitalar do Baixo Vouga, Aveiro and all the parturient previously signed an informed consent. A fluorescence spectrophotometer was used to quantify levels of PAHs (naphthalene, phenanthrene, pyrene and benzo[a]pyrene equivalents) in the tissues collected.

In general, the studied group presented high levels of PAHs equivalents in the placenta (total homogenate fraction) and low levels in the umbilical cord blood. The low molecular weight PAHs (naphthalene and phenanthrene equivalents) measured in placenta presented higher levels than high molecular weight PAHs (pyrene and benzo[a]pyrene equivalents). Considering the county of residence, the highest PAHs levels in placenta were found in parturient from Aveiro, Ílhavo and Albergaria-a-Velha and the lowest in Águeda. Moreover, increased levels of naphthalene and phenanthrene equivalents were associated with mothers' exposure to vehicle exhaust, while high levels of benzo[a]pyrene equivalents were associated with their exposure to tobacco smoke at work. The highest levels of naphthalene, phenanthrene and benzo[a]pyrene equivalents were found in homogenized placenta of mothers who smoked in the third trimester of pregnancy. No significant correlations were found between levels of PAHs equivalents present in biological tissues studied and anthropometric data of newborns, but in general, high PAHs levels were found in newborns groups with low weight, head circumference, and length.

This work provided an overview of the PAHs levels on pregnant woman from the Aveiro district, identifying the main sources of exposure to PAHs. Furthermore, placenta do accumulate PAHs, but is not a complete barrier for those lipophilic compounds, since they seem to cross cell membranes reaching umbilical cord blood and potentially causing adverse effects in the newborns, such as, low weight, length and head circumference values, despite the lack of statistical significance.

Table of contents

List of abbreviations and acronyms

AA-EQS- Annual average of environmental quality standards
ACGIH- American Conference of Governmental Industrial Hygienists
BaP- Benzo[**a**] Pyrene
BCRP- Breast cancer resistance proteins
BMI- Body mass index
BPA- Bisphenol A
CO- Carbon monoxide
CO_2- Carbon dioxide
CYP- Cytochrome P
CYP1A1- Cytochrome P1A1
CYP1A2- Cytochrome P1A2
CYP1B1- Cytochrome P1B1
CYP450- Cytochrome P450
DMSO- Dimethyl sulfoxide
DNA- Deoxyribonucleic acid
E2- β-estradiol
EDC- Endocrine disruptor chemical
FAO - Food and agriculture organization of the United Nations
FF wavelength- Fixed fluorescence wavelength
FSH- Follicle stimulate hormone
GC- Gas chromatography
GC-MS- Gas chromatography-mass spectrometry
Hg - Mercury
HPAHs- High molecular weight polycyclic aromatic hydrocarbons
hPL- Human placental lactogen
HPLC- High performance liquid chromatography
I/O ratios - Indoor/outdoor ratios
IARC- International agency for research on cancer
INE- Instituto Nacional de Estatística
JECFA- Joint FAO/WHO expert committee on food additives
Kg/m^2 - kilogram per square meter
Kow- Octanol/water partition coefficient
LED – Light emitting diode
LOD- Limit of detection
LOQ- Limit of quantification
LPAHs- Low molecular weight polycyclic aromatic hydrocarbons
MAC-EQS- Maximum annual concentration of environmental quality standards
Max - Maximum
MDR1 - Multidrug resistance protein 1
Min - Minimum
MOE- Margins of exposure
mRNA- messenger ribonucleic acid
MRPs- Multi drugs resistance associated proteins
N_{ox}- Nitrogen oxides
O_2- Oxygen
OH-PAHs – Metabolite of polycyclic aromatic hydrocarbons
P53- Protein p53
PAHs - Polycyclic aromatic hydrocarbons
PFA- Perfluoroalkyl substances
PM- Particulate matter
PM_{10} – Particulate matter with 10 micrometer of diameter
PMT- Photomultiplier tube
POPs- Persistent organic pollutants
r - Spearman correlation coefficient

R^2 – R-squared

RBC- Red blood cells

ROS- Reactive Oxygen Species

SD - Standard deviation

SFS- Synchronous fluorescence spectroscopy

SO_2- Sulphur dioxide

TPAHs - Total of polycyclic aromatic hydrocarbons

U-HPLC- Ultra- high performance liquid chromatography

US EPA - United States Environmental Protection Agency

USA- United States of America

VOCs- Volatile organic compounds

WBC- White blood cells

WHO- World health organization

List of Tables

List of Figures

Chapter I: General Introduction

1.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) can be considered persistent organic pollutants in the environment (Meire, Azeredo and Torres, 2007). Natural emissions are related to forest fires, volcanic eruptions, algal and bacterial synthesis, and deposition of organic matter, petroleum seeps and rock erosion that contain petroleum hydrocarbons (Abdel-Shafy and Mansour, 2015). In addition to natural origin sources there may also be PAHs of anthropogenic origin that can affect the environment and human health. Anthropogenic emissions arise from incomplete oil burn or fossil fuels combustion products (Kim *et al*., 2013). Abdel-Shafy and Mansour (2015) divided PAHs into three categories: pyrogenic, petrogenic and biological in the environment. Pyrogenic PAHs can occur under low oxygen levels or anaerobic conditions and high temperatures. These PAHs can occur through incomplete combustion of the motor fuels of vehicles or incomplete combustion of wood in forest fires. The petrogenic process is characterized by crude oil maturation. Most spills of crude oil products happen in the ocean or in fresh water and are associated with the transportation and the storage. The PAHs with the biological production may arise from bacteria and plants synthesis during the organic matter degradation (Abdel-Shafy and Mansour, 2015).

There are anthropogenic sources that can affect the well-being of the humans resulting in carcinogenic, mutagenic and teratogenic effects. The PAHs contact with human can lead to repercussions in short or long term health effects. The principal short term health effects are related with eye and skin irritation and inflammation, nausea and vomit. The principal long term health effects can be cancer, kidney and liver damage, strokes and gene mutation (Kim *et al.*, 2013). Life style can be a potential promoter of PAHs bioaccumulation. Smoking can cause neuro-teratogenic and fetal brain impacts. This can explain future cognitive, emotional and behaviour problems in children (Machado *et al.*, 2014).

PAHs can become bioaccessible and be found in the blood or placenta through different mechanisms like facilitated diffusion, passive diffusion and active transport depending on PAHs intrinsic properties (Chen *et al*., 2014). In the tissues or organs, the PAHs can be metabolized by enzymes and then can be accumulated or excreted. The developing fetus and infants can be more sensitive to environmental toxicants than healthy adults. Maternal exposure to PAHs can be a fetal growth reduction factor

(preterm delivery and low birth weight), head circumference reduction and intrauterine restrictions *(*Chen *et al.*, 2014).

1.2 PAHs in environment

PAHs structure can be influenced by the source (Kim *et al.*, 2013). PAHs are organic compounds constituted by hydrogen, carbon and benzene rings (Kim *et al.*, 2013). PAHs classified as low molecular weight have 2 or 3 benzene rings like naphthalene and phenanthrene equivalents), respectively. PAHs with high molecular weight have 4 (pyrene equivalents), 5 (benzo[**a**]pyrene or BaP equivalents), 6 (Benzo[**ghi**]-perylene equivalents) or 7 (indeno[1,2,3-**cd**]pyrene equivalents) benzene rings (Urbancova *et al.*, 2017). There are some characteristics attribute to the PAHs according the benzene rings. PAHs with fewer benzene rings (2 or 3) are usually found in volatile phase and PAHs with more benzene rings (4 or more) usually are found in particulate state (Mohanraj, Dhanakumar and Solaraj, 2012). Larger PAHs compounds (4 or more benzene rings) were found at higher concentrations in autumn and winter compared with those from spring and summer. These occurrences happen because the PAHs aging process relays in volatilization of finer particles and then the condensation onto bigger particles. Another explanation for this event is that PAHs and other pollutant mixtures could merge with long-range transported aerosol in cold seasons (winter and autumn). Lai *et al.*, (2011) suggested that higher PAHs concentrations are observed in winter and arise from coal combustion and forest fires. The gas phase PAHs are dominant in days with high temperatures (summer time or tropical regions) and particulate phase PAHs occur more frequently at low temperatures (winter time or arctic regions). The adsorption of PAHs from gas phase to the particulate phase can be related with humidity (Kim *et al.*, 2013). Humidity has no direct significant correlation with the several PAHs species properties. However, humidity can be related with other parameters like wind speed and rainfall. Chrysene equivalents (3 benzene rings) and BaP equivalents (5 benzene rings) have a positive correlation with wind speed and rainfall. Humidity can be a non-direct physical-chemical parameter to promote PAHs appearance like BaP and chrysene equivalents. PAHs can occur through different forms (volatile and particulate) in different times of the year (winter or summer) that can lead to a different deposition type of atmospheric particulate matter (PM). Wet deposition of atmospheric PM is carried out by fine particles in the majority of cases. In the

environment, the dry depositions of atmospheric PM are more common in PAHs with high molecular weight like BaP (Kaupp and Mclachlan, 1999; Zhang, Zhang and Wang, 2009). The fate of PAHs in the environment can fluctuate thought different factors: physicochemical reaction, interactions with other pollutants, PAHs degradation and dry and wet deposition (Kim *et al*., 2013). Most of PAHs are assimilated with other gaseous pollutants as SO_2, N_{ox}, CO, ozone (Kampa and Castanas, 2008). The gaseous pollutants can occur through fossil fuels combustion. Volatile organic compounds (VOCs) are the larger part of the gaseous pollutants. In this group are present species of organic compounds like PAHs (Kampa and Castanas, 2008).

Usually, in the environment pollutant mixtures have PAHs metabolites in their constitution. They frequent regroup with other PAHs or other xenobiotics depending on their composition in the soil, oil in water and sediments or volatile state (Abdel-Shafy and Mansour, 2015). PAHs can be more persistent in environment than in human tissues. PAHs compounds are ubiquitous contaminants formed in thermal decomposition of organic molecules (WHO, 2010; Wang *et al*., 2013).

There are several procedures for treating the contaminated soil and usually are a combination of an *in situ* treatment, on-site treatment and bioreactors (Wilson and Jones, 1993). The fate of PAHs is determined by soil removal (biodegradation, leaching or hydrolysis) and deposition process. The PAHs soil removal process is suitable for the 2 or 3 benzene rings PAHs mainly with mechanisms of leaching, hydrolysis or volatilization (Wilson and Jones, 1993). PAHs with more than 4 rings usually are more difficult to remove than the 2 or 3 benzene rings structure compounds. In addition, the high molecular weight PAHs are not prone to leaching to underground water and usually are available for plants uptake. The major mechanism of PAHs removal from the soil is biodegradation. Biodegradation consists in biological action of microorganisms, where bacteria, fungi, and yeasts are able to degrade PAHs (Meire, Azeredo and Torres, 2007). This degradation can help remove contaminants from the environment. Phytoremediation is other procedure to reduce or eliminate the abundance of PAHs in the environment (Glick, 2003).

The removal of organic pollutants from the water can occur through several processes like destructive oxidation through ozone, hydrogen peroxidase, monogenesis oxide, or porous solids. The adsorption technique is used for the removal of hydrophobic organic compounds at water the activated carbon, bentonite (Smith and

Galan, 1995), fractured chalk (Wefer-Roehl et al. 2001), wood (Boving and Zhang, 2004) or Leonardite (Zeledón-Toruño *et al.*, 2007).

Biomonitoring of PAHs in the environment and in human tissues helps to evaluate the stage of population contamination and understand the severity of the organic compound situation (Glick *et al.*, 2003; Machado *et al.*, 2014).

1.3 Polycyclic Aromatic Hydrocarbons threshold levels

It is difficult to create a standard PAHs level for tissues because there are several studies that use different measurements techniques and unities (Sexton *et al.*, 2011). Biomonitoring sediment, water and air can be an useful technique to evaluate PAHs in environment and promote the well-being of the humans (Srogi, 2007). Several institutions and entities have tried to categorize PAHs due to cases of environmental contamination and consequential effects on human health (Conti *et al.,* 2012).

Benzo[**a**]pyrene is used as a marker in food, air as well as in water because of the human carcinogenic effects. The inhalation concentration for BaP equivalents should not pass 0.032 µg/day because of their carcinogenic characteristic (WHO, 2010). U.S. Environmental Protection Agency (EPA) states as maximum air level of 0.1 µg/m^3 for benzo[**a**]anthracene 0.2 µg/m^3, benzo[**b**]fluoranthene, benzo[**k**]fluoranthene and chrysene. 0.3 µg/m^3 for dibenzo[**a,h**] anthracene and 0.4 µg/m^3 for indenol[1,2,3-**c,d**]pyrene (WHO, 2010). Most studies reveal inconclusive to determine a maximum PAHs level in ambient air. The Directive 2004/107/CE15 of 2004 states a maximum BaP equivalents level for the total content in the PM_{10} fraction averaged over a year of 1 ng/m^3.The American Conference of Government Industrial Hygienists allow 200 µg/m^3 benzene-soluble coal tar pitch fraction for a limit value in the workplace. The American National Institute for occupational safety and health has a recommended exposure limit of 100 µg/m^3 for coal pitch volatile agents in close spaces. Occupational safety and health American administration have an exposure limit of 200 µg/m^3 of benzene soluble coal tar pitch fraction. Major cities in India have a range of 190.96-672 ng/m^3 of in the air despite the standard limit of 5 ng/m^3 for air (Singh *et al.,* 2008).

As aforementioned, PAHs could be found in preparation, conservation and storage, mostly. PAHs high concentrations were found in some products like: dried fruits, olive pomace oil, smoked fish, grape seed oil, smoked meat products, fresh mollusks, spices/sauces and condiments (Table 1-1). FAO/WHO expert Committee on

food additives (JECFA) in 2005 projected a risk on PAHs and established margins of exposure (MOE) for PAHs as a basis for genotoxic and carcinogenic compounds. The margins were established for benz[**a**]anthracene, benzo[**b**]fluoranthene, benzo[**j**]fluoranthene, benzo[**k**]fluoranthene, benzo[**g,h,i**]perylene, chrysene, cyclopenta[**c,d**]pyrene, dibenz[**a,h**]anthracene, dibenzo[**a,e**]pyrene, dibenzo[**a,h**]-pyrene, dibenzo[**a,i**]pyrene, dibenzo[**a,l**]pyrene, indeno[1,2,3-**cd**]pyrene and 5-methylchrysene. JECFA recommends the Benzo[**c**]fluorine equivalents analyses. Principally in products containing fats and oils, smoking or drying processed foods (fish and fishery products) because of the high risk of contamination (UE Directive 2015/1787 of 2015). In the case of mollusks and fish in straight contact with sediments or feeding on organisms in contact with sediments, some extra concern may be raised. PAHs in sediments may increase in time, due to deposition processes and constitute a risk to the environment, but also to humans (Hontela *et al.,* 1992).

Table 1-1- Maximum PAHs levels in different food products according to the EU directive 2015/1787 from 2015.

	Benzo[a]Pyrene (µg/kg)	**ΣPAHs (Benzo[a]Pyrene, Benzo[a]anthracene, benzo[b]fluoranthene, chrysene)** (µg/kg)
Oils and fats (except butter and coconut oil)	2	10
Cocoa beans and related products	50 (since 2013)	30 (since 2015)
Coconut oil (human consumption)	2	20
Smoked meat and related products	2 (since 2014)	12 (since 2014)
Smoked *Sprattus sprattus*, bivalves (fresh or frozen), fresh meat	5	30
Smoked bivalves	6	35
Cereals and related products	1	1
Infant formulas	1	1
Diet products	1	1

PAHs are classified as priority pollutant in water by the Directive 2008/105/CE of 2008. The water suitable for consumption can be other source of PAHs ingestion. According to WHO (2010) and IARC (2010) there are PAHs compounds found in water like fluoranthene, benzo[**a**]anthracene, benzo[**b**]pyrene, benzo[**a**]pyrene, benzo[**k**]fluoranthene, dibenz[**a,h**]anthracene, benzo[**g,h,i**] perylene, indeno[1,2,3-**cd**]pyrene. These compounds should not pass a standard level of 0.1µg/L. According to Decreto-Lei n^{o} 306/2007 of 2007, the standard level for BaP in water is 0.01 µg/L and 0.1 µg/L for the total concentration of benzo[**b**]fluoranthene, benzo[**k**]fluoranthene, benzo[**g,h,i**]perylene and indeno[1,2,3-**c,d**]pyrene. In Table 1-2 is presented the maximum PAHs levels in inland and other waters according the Directive 2008/105/CE of 2008. US EPA classified the 0.2 µg/L as the maximum for benzo[**a**]pyrene in water. Higher concentrations than 0.2 µg/L could be harmful to humans. The major cities in India reach levels of PAHs in water up to 65.85 µg/L and the standard limits are 0.2 µg/L (Singh *et al.*, 2008).

Table 1-2- Maximum PAHs levels (µg/L) in water. The analysis was made by AA-EQS and MAC-EQS (inland and other waters) parameters second Directive 2008/105/CE from 2008.

Substance Name	AA-EQS Inland surface waters (µg/L)	AA-EQS other surface waters (µg/L)	MAC-EQS Inland surface waters (µg/L)	MAC-EQS other surface waters (µg/L)
Anthracene	0.1	0.1	0.4	0.4
Benzene	10	8	50	50
Fluoranthrene	0.1	0.1	1	1
Naphthalene	2.4	1.2	Not applicable	Not applicable
Benzo[a]pyrene	0.05	0.05	0.1	0.1
Benzo[b]fluoranthene Benzo[k]fluoranthene	0.03	0.03	Not applicable	Not applicable
Benzo[g,h,i]perylene Indeno[1,2,3-cd]pyrene	0.002	0.002	Not applicable	Not applicable

1.4 Low Molecular Weight PAHs

Low molecular weight PAHs (LPAHs) are compounds with 2 or 3 benzene rings. The principal human exposure sources of LPAHs are coal tar, gasoline, diesel fuel, paper burning and cigarette smoke (Lai *et al.*, 2011; Kim *et al.*, 2013). This can be related with less structurally stable molecules. The photolysis of LPAHs is more effective because they are more bioavailable in matrices and have a longer exposure time to sunlight. These compounds tend to be in the vapour phase, are highly degradable in water and have a low adsorption in sediment (Figure 1-1) (Kim *et al.*, 2013; Abdel-Shafy and Mansour, 2015).

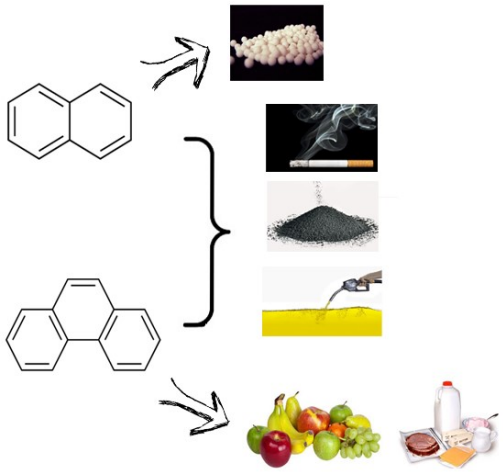

Figure 1-1 - Representation sources of LPAHs (Naphthalene equivalents with 2 benzene rings and phenanthrene equivalents with 3 benzene rings) (Adapted from: American Energy News, 2017; Elken, 2017; Healthy preschoolers, 2017; Mariajoaoalmeida, 2017; Sigma-Aldrich, 2017b; Sigma-Aldrich, 2017c; Time, 2017; Wixstatic, 2017).

Naphthalene equivalents are bicycle aromatic hydrocarbons with low molecular weight (Kim *et al.*, 2013; Abdel-Shafy and Mansour, 2015). In the human body, naphthalene equivalents proceed to hepatic metabolism where they are oxidized to other metabolites like alpha-naphtol. Alpha-naphtol is an oxidative metabolite that can lead to methemoglobinemia formation and hemoglobin oxidation. Oxidized hemoglobin is desaturated with an outcome of Heinz bodies and hemolysis of red blood cells (RBC). The hemolysis or methemoglobinemia take place in 1 or 2 days and when reach a peak in 3 to 5 days the anemia resulting of RBC hemolysis (Sudakin, Stone and Power, 2011). In human biomonitoring studies, metabolized naphthalene in 1-

hydroxynaphthalene and 2-hydroxynaphthalene were observed in the urine of USA and Europe adults and children (Preuss, Angerer and Drexler, 2003) . In rhesus macaques it was possible to report important information effects of naphthalene in the respiratory tract that affect epithelium cells and lungs mainly (Li *et al.*, 2016).

The National Toxicology Program in the USA (1992-2000) related pulmonary alveolar/bronchiolar adenomas induced by cytotoxic effects of naphthalene equivalents mechanism in cells. The newborns are more sensitive to naphthalene equivalents exposure that adults because of their thinner skin and due to the decrease stores if reduced glutathione. Prenatal toxicity can be a result of naphthalene equivalents levels cross over the placenta (Sudakin, Stone and Power, 2011). Madhavan and Naidu (1995) related a possible LPAHs species transaction from the mother to the fetus as PAHs residual levels detected in human placenta, umbilical cord blood, maternal blood and human milk. In Shengsi Islands, the highest PAHs concentrations found in umbilical cord serum from 120 mothers and newborn pairs were of naphthalene equivalents (of the 16 PAHs species analyzed). These compounds are classified as possible carcinogenic by EPA, mutagenic and teratogenic to humans (Sudakin, Stone and Power, 2011) . The International Agency for Research on Cancer (IARC, 2010) classifies in terms of effects the different PAHs species in 4 groups. (1) 119 PAHs are considered carcinogenic to humans; (2a) 81 PAHs are "Probably" carcinogenic to humans; (2b) 292 PAHs species are "Possible" carcinogenic to humans; (3) 505 species are not possible to classify 505 PAHs species; (4) Just 1 PAHs species is "Probably" not carcinogenic to human. Naphthalene equivalents is a "possible" carcinogenic to human. Additionally to this classification, Farhadian *et al.* (2012) created some important key points of PAHs classification in groups to add as those described by IARC. In a first approach it is important to investigate the kinetics, PAHs formation and mechanism of mutagenic/carcinogenic/teratogenic activities, followed by PAHs examination in food and biomonitoring human exposure to PAHs.

Naphthalene equivalents are present in the environment in tobacco smoke, coal tar and mothballs (Kim *et al.*, 2013; Sudakin, Stone and Power, 2011). Mothballs are 99.9% naphthalene equivalents and the weight can fluctuate from 0.5g to 5g. However, they are usually sold in 396 g cases and can release 200 $\mu g/m^3$ in an 1-year period. National Pesticide Information Center in the USA in 2006 had 769 reports of naphthalene equivalents exposure incidents. 60% of the cases were associated with misapplication of the compound. Color and ball shape can induce a wrong perception in

small children because it is usually mistaken for a toy ball. These can lead to some cases of naphthalene equivalents ingestion (Sudakin, Stone and Power, 2011).

In an investigation regarding urban, rural and intermediate place of residence in France was possible to observe high concentration were of low molecular weight PAHs. The highest concentration found was of naphthalene equivalents with 53.7 ng/m^3 and phenanthrene equivalents with 26.2 ng/m^3 (Annamalai and Namasivayam, 2015). Phenanthrene equivalents arise from biomass, wood combustion, and food such as meat, fruit, dairy products, oils, and fats. Concentrations in these products ingested by humans can be up to 18.18µg / kg phenanthrene equivalents (Martorell *et al*., 2010; Mohanraj, Dhanakumar and Solaraj, 2012). There are other sources like pesticides and resins formation (Kim *et al.*, 2013; Abdel-Shafy and Mansour, 2015). Phenanthrene equivalents have slow photolysis degradation because of the molecular stability of this compound. In the atmosphere phenanthrene equivalents tend to form a molecular complex (Abdel-Shafy and Mansour, 2015).

Phenanthrene and anthracene equivalents are the smaller PAHs types classified as "possible non-carcinogenic" polycyclic aromatic hydrocarbons by IARC (IARC, 2010). Jung *et al.* (2013) study present a several PAHs concentrations exposure - including phenanthrene equivalents - to rat's liver. That exposure result in transcriptomic changes in rat's liver tissue. More studies are necessary to clarify genotoxic effects of phenanthrene equivalents in human tissues (Kim *et al*., 2013).

Phenanthrene-type PAHs have higher concentrations in winter and urban areas (Mohanraj, Dhanakumar and Solaraj, 2012). It is possible that phenanthrene equivalents cause teratogenic effects (Kim *et al*., 2013). It was measured PAHs species in placenta of children with neural tube defect and children without neural tube defect. The children with neural tube defects had higher phenanthrene and fluorene equivalents levels than the children without the neural tube defects (Ren *et al*., 2011). Both of these species have three benzene rings (Kim *et al*., 2013).

1.5 High Molecular Weight PAHs

The HPAHs have a low vapour pressure, less aqueous solubility and less photo-sensitivity than LPAHs. Because of HPAHs stability, most species are found in the particulate phase. It is possible to found these PAHs in the environment due to natural

and anthropogenic sources. Natural sources include volcano and forest fires, whereas anthropogenic sources include commercial and industrial combustion, road transport and metal production (Figure 1-2) (Zhang, Zhang and Wang, 2009; Kim *et al*., 2013).

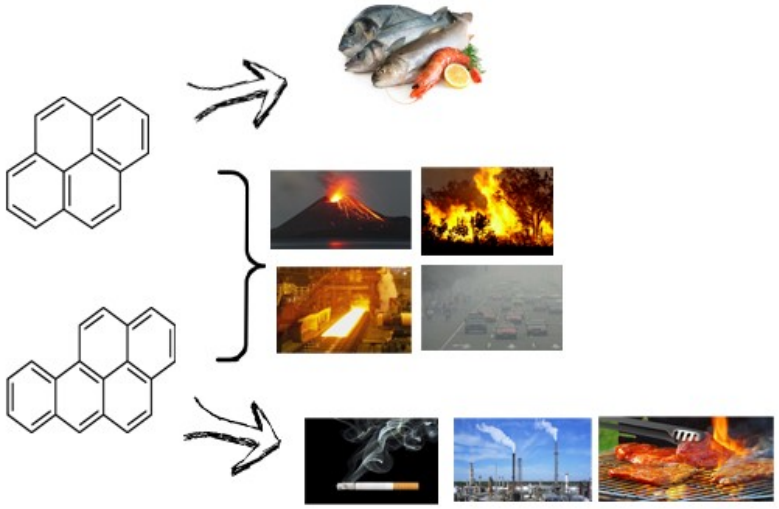

Figure 1-2 - Representation sources of High molecular weight polycyclic aromatic hydrocarbons (Pyrene equivalents with 4 benzene rings and benzo[a]pyrene equivalents with 5 benzene rings) (Adapted from: Amazon, 2017; Cduarouca, 2017; Global Transport Knowledge Practice, 2017; Mundo educação, 2017; Organizar a casa, 2017; Sigma-Aldrich, 2017a; Sigma-Aldrich, 2017d; Scheererbearing, 2017; Skillplus, 2017; Time, 2017).

Pyrene equivalents are high molecular weight compounds with four benzene rings and classified as non-carcinogenic to human (IARC, 2010). Generally, pyrene equivalents enter the human body by ingestion. In Shengsi Islands (China), the majority of ingested protein is obtained through aquatic products (Yin *et al*., 2016). Umbilical cord serum samples collected from parturient living in the East China Sea presented high levels of pyrene equivalents. Feeding choices (fish consumption) can be a source of human contamination by pyrene equivalents. Yin and co-authors (2016) found a possible correlation between pyrene equivalents and reproductive hormones like estrogens' hormones. The PAHs exposure negatively affects β-estradiol (E2) and positively affects FSH in umbilical cord blood. So, it is possible that PAHs as pyrene equivalents act as a type of endocrine disruptor chemical (EDC).

Benzo[**a**]pyrene or BaP equivalents are compounds composed by five benzene rings, considered large size PAHs and usually is found in environment adsorbed to particulate phase xenobiotics. BaP equivalents are metabolized in human organism. In metabolization phase, nonpolar PAHs are transformed into phenols and diols. Phenols and diols bind to DNA forming PAH-DNA adducts. This binding promotes genetic

mutations and can be associated with different cancer types (Kim *et al*., 2013; Machado *et al*., 2014). The p53 protein mutation can be related to the skin and lung cancer. These mutations are caused by xenobiotics such as BaP equivalents (Abdel-Shafy and Mansour, 2015).

Still considering long term effects, BaP equivalents can be considered was the first studied specie of PAHs that had carcinogenic, genotoxic and teratogenic effects in human (Abdel-Shafy and Mansour, 2015). 1-Hidroxypyrene and BaP equivalents were found in the urine of smoker and passive smoker parturient. These BaP equivalents and 1-hydroxypyrene have a positive correlation with cotinine found in parturient. This correlation can be an explanation to neuroteratogenic effects like brain damage, cognitive problems, emotional and behavioural effects in newborns and children (Machado *et al*., 2014). Low birth weight, premature delivery, heart malformations are some of the birth outcomes related to PAHs exposure. EPA and IARC describe BaP equivalents a human carcinogenic (IARC, 2010; Kim *et al*., 2013; Machado *et al*., 2014). In contact with the skin in a short term effect, BaP equivalents are a skin irritant. Most of the BaP equivalents exposures are related to tobacco smoke but there are other sources like vehicle exhaust, barbecued, roasted, fried and smoked meat and industrials smoke (Choi *et al*., 2012; Jedrychowski *et al*., 2013; Kim *et al*., 2013; Abdel-Shafy and Mansour, 2015).

1.6 Kinetics of PAHs in the human organism

There are several ways of PAHs adsorption on the body. Different PAHs species have different modes of action and can bioaccumulate in diverse organs. Usually, PAHs concentrations are higher in the liver because of his filtration and detoxification functions (Meire, Azeredo and Torres, 2007; Sexton *et al*., 2011 and Kim *et al*., 2013).

Inhaled PAHs can be adsorbed by bronchial epithelial cells, skin cells and intestine cells. PAHs stay in ciliated mucus or penetrate through bronchial epithelium cells where they are metabolized. The absorption occurs also in other pathways, like ingestion and skin absorption (Meire, Azeredo and Torres, 2007; WHO, 2010; Kim *et al*., 2013). PAHs distribution through the body can be fast. Most PAHs species are lipophilic. This characteristic allows PAHs to have an easy passage through biologic membranes (WHO, 2010). Factors like age, gender, body fat percentage, metabolism of xenobiotics, physical characteristics (body mass index) and lifestyle (smoking, alcohol consumption,

physical activity) can influence the PAHs metabolism. The excretion can occur through urine or faeces. Species of LPAHs are excreted via urine mostly and high molecular weight PAHs species can be excreted via feces. However, there are PAHs that accumulate in the body (Urbancova *et al*., 2017).

Biotransformation is a process where the conversion of a xenobiotic into other compounds occurs inside organisms. PAHs biotransformation process is based on the lipophilic metabolite transformation in a hydrophilic metabolite. Transformation main goal is to make an easier excretion of the metabolites. PAHs biotransformation in phase I oxidation metabolism by CYP450 (cytochrome P450 family) monooxygenases to reactive epoxide intermediates followed by reduction or hydrolyzes like urinary PAHs metabolites (OH-PAHs). In phase II metabolism, a conjunction reaction of OH-PAHs with glucuronic acid or sulphate occurs to enhance water solubility (Choi *et al*., 2012).

The induction of CYP (cytochrome) from de Ah receptors is related with the PAHs excretion. In PAHs metabolism there are two types of key enzymes/groups: cytochrome P450 family and epoxide hydrolase. In the cytochrome P450 family the CYP1A1, CYP1A2, and CYP1B1 are the key enzymes more produced to metabolize PAHs. There are three principal mechanisms on activation of PAHs for toxic intermediates in metabolism:

- The most common (dihydro)diol-epoxides formation through enzymatic oxidation reactions resulting in hydrolysis;
- Via radical cation formation;
- O-quinone pathway. The CYPs metabolize PAHs in several Quinones (Meire, Azeredo and Torres, 2007; WHO, 2010; Choi *et al*., 2012).

There are PAHs capable of binding with macromolecules (proteins and nucleic acid) and before phase I became reactive metabolites causing damage. This damage might include:

- Mutagenic effects: DNA adduct formation blocks DNA from replicate and induces base and nucleotide excision repair activities. These repair activities can induce errors in DNA replication. These miscalculations can be fixed mutations after cell division. Pathological abnormalities (leiomyomas) in vagina, cervix, and uterus are related with mutagenic effects in mammal species (Annamalai and Namasivayam, 2015; Abdel-Shafy and Mansour, 2015);

- Carcinogenic effects: some alterations in DNA can lead to lack of control of cell formation that leads to the formation of cancers like adrenal and thyroid tumors and mammary adenocarcinomas (Abdel-Shafy and Mansour, 2015; Annamalai and Namasivayam, 2015);
- Teratogenic effects: PAHs teratogenic effects like low birth weight, premature delivery and heart malformations of the fetus can be connected with a possible human exposure (Abdel-Shafy and Mansour, 2015).

1.7 Challenges in PAHs chemical analysis

Several studies try to create a best way for PAHs chemical analysis. The fixed wavelength fluorescence (FF) measurement technique refinement can generate a premium methodology with some important characteristics like low cost, high number of samples analysis in a short time period, a sensitive method and easy to understand by the user. Usually for PAHs measurement is used gas chromatography-mass spectrometry (GC-MS) (Sexton *et al*., 2011; Yu *et al*., 2011), high performance liquid chromatography (HPLC) with fluorescence detection (Singh *et al*., 2008; Chen *et al*., 2014; Machado *et al*., 2014), synchronous fluorescence spectroscopy (SFS) and fixed wavelength fluorescence (FF) (Aas *et al*., 2000).

Gas chromatography-mass spectrometry is an analytical method used in the detection of several volatile and semi-volatile organic compounds. Some detections of GC-MS technique are:

- Quantification and identification of volatile or semi-volatile PAHs in mixtures of several compounds and;
- Molecular weight, structural determination and possible composition of unknown PAHs (Hites, 1997).

EPA uses GC-MS techniques to quantify the pollution of the drinking water and waste water. Other entities in the field of pharmaceutical or forensic sciences use GC-MS for PAHs and drugs determination in blood or urine. In food industry it is used for the product quality control. GC-MS jet separator is one important commercial GC carrier gas separator that takes advantages of the differences in diffusibility among the carrier gas and the organic compound (like PAHs). Usually, the carrier gas has high diffusion coefficient and the organic pollutants have a lower diffusion coefficient. The carrier gas is sprayed over a wide solid angle and the high molecular weight PAHs are

sprayed in a much narrower angle and go straight to vacuum region. It is collected the middle section of a solid angle with a skimmer. This section is passed in to a mass spectrometer and PAHs with high molecular weight are separated from the carrier gas that is removed from a vacuum pump. The major limitation of GC-MS methodology is that only compounds exceeding 10^{-10} torr of vapour pressure are analyzed. This fact restrict the qualitative accuracy (Hites, 1997).

Chromatography is a separation method with sensitive detection that allows micro quantities analysis of complex matrices material (Lingeman *et al*., 1985). HPLC was developed for clinical, pharmaceutical, environmental and food analysis (Gámiz-Gracia *et al*., 2009). Adsorption onto surfaces, partition coefficients in systems of two non-homogeneous mixtures, ionic solutes interactions with ion-exchanges site surface or molecular size are the principal characteristics of the liquid chromatography analysis. The quantum yield of fluorescence of these chemicals is frequently low in polar solvents, which is the principal disadvantage of this methodology. The HPLC detection occurs in a fluorimeter equipped with a flow cell (on-line detection) or fixed wavelength fluorescence (FF) (off-line detection). The selection of fixed fluorescence wavelength the fluorimeter, filters or monochromators are essential for the sample analysis. Excitation and emission radiation intensity have a higher sensitivity with filters. The monochromator promotes enhancement and selectivity of PAHs detection in urine or serum samples better than filters (Lingeman *et al*., 1985).

The fluorescence measurements are performed by an excitation spectrum. That excitation spectrum is achieved by scanning the excitation monochromator while setting the emission monochromator at an optical wavelength. An emission spectrum monochromator set at an appropriate single-excitation wavelength (Li *et al*., 2011). Two polarization filters (excitation/emission) are necessary for anisotropy measurements. The monochromator can be adjusted to the wavelength selected. Spectrofluorimeters apply diffraction grating monochromators for the incident and fluorescent light incitation. Filter fluorimeters make use of filters to isolate light. Spectrofluorimeters and fluorimeters follow the same scheme for detecting the sample fluorescence. The light (LED, laser or lamp) came from an excitation source and passes through a filter or a monochromator to hit the sample. Photons influence on the excitation monochromator witch discriminatively transmits light in a confine range focusing in the specified excitation wavelength. The transmitted light cross adjustable slits that regulate magnitude and resolution by restrict the range of transmitted light. The filtrated light

goes into the cell of the sample creating fluorescence emission by fluorophores within the sample. The fluorescence is measured at a 90º angle in order to not take place interference. Emitted light passes in a confine range centred above the wavelength and gets out through adjustable slits and lastly enters the photomultiplier tube (PMT). The amplifier signal forms a voltage that is proportional to the emitted intensity measurement. Could occur some noise derived from PMT. The detector can be single channelled or multichannel. Single channelled measures one wavelength and multi-channel measures several wavelengths at one time (Al-Rawashdeh, 2012).

SFS (Synchronous fluorescence spectroscopic) is the technique low cost and simpler than HPLC. SFS is obtained by the scan of both excitation/ emission monochromators simultaneously (Lin, Cormier and Torsella, 1996; Li *et al*., 2011). Vo-Dinh (1978) define some principal characteristics of this technique: (1) Confine spectral bands that stem from the multiplication of two increasing and/or decreasing functions, simultaneously; (2) Emission spectra's simplification considering that conventional fixed spectrofluorimetry and only it is possible to rise the intensity of all of the emission bands at some time, the synchronous technique enable the higher peaks to be rise selectively by use of a suitable wavelength; (3) Reduction of the spectral range. An analytical point of view all spectrum are not interesting and some details may not be considered.

Fixed wavelength fluorescence (FF) detection is a simpler method, allows measuring PAHs exposure on a large number of samples and at a lower cost than HPLC and GC-MS techniques. It is a highly sensitive technique and it is possible to obtain positive results with low quantities of sample. These advantages make this technique suitable for clinical analyses (Aas *et al*., 2000). This technique was used in this thesis and in other studies (e.g. Gravato and Santos, 2003). In order to understand if crude oil was bioaccumulated in cod fish was used fixed wavelength fluorescence (FF) measurements to determine PAHs concentrations in bile samples (Aas, Beyer and Goksøyr, 2000). Oliva *et al*. (2010) covered a larger PAHs concentration range in order to collect bigger information about the PAHs concentrations in *Solea senegalensis* liver samples.

The major disadvantage of fixed wavelength fluorescence and synchronous fluorescence spectrometry can be considered overlapping problems. Overlap can confuse the results in the presence of all spectrums and the emission of other PAHs in samples. The resolution of the mixture spectra it is possible to overcome but is very

time consuming and the equipment that is used for that aim is very expensive. So, in order to overstep the problem other techniques have been developed as the combination of synchronous fluorescence spectrometry with chemometrics or using DMSO as a solvent in PAHs measurement. The DMSO reduces the interferences of the different spectra suppressing the background noise and band-narrowing the specific PAHs -type that it is measured. In Figure 1-3 it is possible to observe how naphthalene, phenanthrene, pyrene and BaP equivalents were localized in the spectrum and the possible overlap (Li *et al*., 2011).

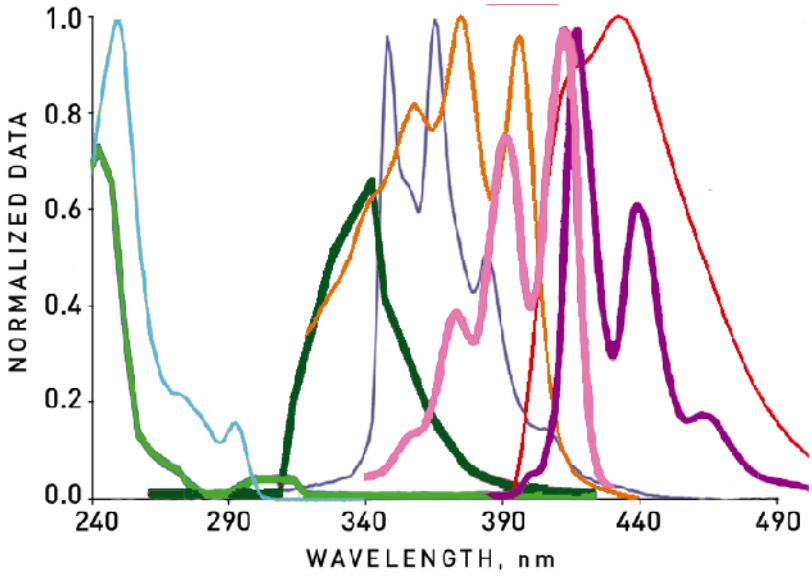

Figure 1-3 - Naphthalene (green) Phenanthrene (blue), Pyrene (pink) and Benzo[a]pyrene (red) Wavelength (nm) representation. The lighter lines are the emission wavelength, the darker lines represent the excitation wavelength (Adapted from Li et al., 2011).

1.8 Human matrices as biomarkers of exposure to PAHs

Human health can be influenced by life style factors that include medication, smoke, drug abuse, alcohol consumption and environmental sources. Xenobiotics enter the body and bioaccumulate in different organs because of the different metabolization processes associated. Organs, tissues and cell biomonitorization can be used to measure the target chemicals. It is important to establish detection limits for corresponding organs, tissues or cell analyses for the different analytical techniques (Myllynen, Pasanen and Pelkonen, 2005; Esteban and Castaño, 2009) . Different human matrices analyzed can provide information about several persistent organic pollutants (Table 1-3). Some studies revealed that PAHs are able to cross from mother to fetus thought the

placenta barrier (Madhavan and Naidu, 1995; Sexton *et al*., 2011; Machado *et al*., 2014; Urbancova *et al*., 2017).

Table 1-3 - Range of mean concentration of TPAHs or Phenanthrene, Pyrene, Benzo[b]fluoranthene, 1-hydroxypyrene and BaP in biological samples from world population.

COUNTRY	TISSUES	MEASUREMENT	PAH	MEAN(SD)	REFERENCE
China (Shengsi Islands)	Umbilical cord serum	GC analysis	TPAH*[1]*[2]	218.36(173.84)ng/mL	Yin *et al*., 2016
China (Hong Kong)	**(1)** Maternal Serum **(2)**Umbilical cord serum	HPLC	Pyrene	**(1)**163.24±145.22 ng/mL **(2)**103.88±93.28ng/mL	Chen *et al*., 2014
USA (Texas)	**(1)** Cord blood **(2)**Maternal blood	GC-MS	TPAH*[1]*[3]	**(1)** 12.8 ng/mL **(2)**5.5ng/mL	Sexton *et al*., 2011
Brazil (Porto Alegre)	**(1)** Urine **(2)**Amniotic fluid **(3)**Umbilical cord blood	HPLC	1 e 2- 1-hydroxypyrene 3-BaP	**(1)** 0.15 (9.63) ng/mL **(2)**0.67(1.39) ng/mL **(3)**1.13 (1.99) ng/mL	Machado *et al*., 2014
China (Beijing)	Placenta	GC-MS	TPAH*[5]	9.30(575)ng/g fat	Yu *et al*., 2011
USA (New York)	Placenta	GC-MS	Benzo[**a**]pyrene	2.37 ng/g	Perera *et al*., 2009
India (Lucknow)	Placenta	HPLC	Benzo[**b**] fluoranthene	Median (250.27µg/g)	Singht *et al*., 2008
Ukraine (Kyiv and Dniprodzerzhinsk)	Placenta		PAH*[6]	Median (7.36 µg/g)	Gladen *et al*., 2000
Poland (Lodz and Legnica districts)	Urine	GC-MS	**(1)** Phenanthrene **(2)**Pyrene	**(1)** 3050(2580)ng/mL **(2)**770(600)ng/mL	Polanska *et al*., 2014
Czech Republic (Karvina and Ceske Budejovice)	Urine	U-HPLC	T OH-PAHs *[4]	5.3 ng/mL	Urbancova *et al*., 2017

*[1] Naphetalene, Acenaphtene, Anatracene , Phenanthrene, Fluorine, fluoranthene, pyrene,
*[2] Biphenyl, Dibenzofluorane, Dibenzothiophene.
*Acenaphthylene, benzo[**a**]anthrancene, chrysene, benzo[**b**]fluoranthene, Benzo[**k**]fluoranthene, benzo[**a**]pyrene, indeno[1,2,3-**c,d**]pyrene, dibenzo[**a,h**]anthracene and Benzo[**g,h,i**]perylene.
*[4] naphthalene-1-ol, phenanthrene-1-ol, pyrene-1-ol, fluorine-1-ol; naphthalene-2-ol, phenanthrene-2-ol, phenanthrene-3-ol, phenanthrene-4-ol, benzo[**a**]pyrene-3-ol; crysene.4-ol; benzo[**a**]pyrene-7-ol.
*[5] Acenaphthylene, benzo[**a**]anthrancene, chrysene, benzo[**b**]fluoranthene, benzo[**a**]pyrene, indeno[1,2,3-**c,d**]pyrene, dibenzo[**a,h**]anthracene and Benzo[**g,h,i**]perylene , Acenaphtene, Anatracene , Phenanthrene, Fluorine, fluoranthene, pyrene.
*[6] Chrysene, benzo[**b**]fluoranthene, benzo[**a**]anthrancene, benzo[**a**]pyrene, indeno[1,2,3-**c,d**]pyrene, dibenzo[**a,h**]anthracene and Benzo[**g,h,i**]perylene

The matrices are expelled from the body, so it is not necessary to damage or invade the physical interagency to collect them. However, human milk, placenta, umbilical cord, placenta membrane, urine and even blood are examples of excretions, tissues, organs and membranes used as biomarkers of exposure biomonitorization studies related with PAHs contamination, accumulation and excretion in humans. These are the most used because of their non-invasive characteristics (Esteban and Castaño, 2009; Sexton *et al*., 2011; Yu *et al*., 2011; Machado *et al*., 2014). Non-invasive samples are gathered outside the body so; the organism integrity is not invaded or destroyed. Blood can be classified as non-invasive matrix when removed from umbilical cord or as invasive matrix when it is taken by the mother blood vessels (Esteban and Castaño, 2009) (Figure 1-4).

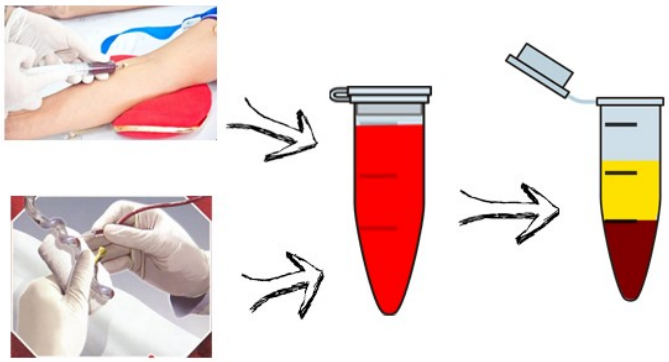

Figure 1-4 - Invasive (intravenous) and non-invasive (umbilical cord) blood collect. Separation blood cells from plasma (Adapted from: Awebic, 2017; Clipartpanda, 2017; Clker, 2017; Culturamix, 2017).

Samples collected from humans are donated to the science by the owner. All the ethical issues must be discussed and analyzed in order to cause effects no impairment in human health (psychologically and physically). Blood is a tissue that contacts with all organs inside the organisms and is used regularly in toxicology studies (Esteban and Castaño, 2009).

1.8.1 Placenta as a biomarker of PAHs exposure

In human toxicology studies, placenta can be used as a matrix after expelled in during parturition. Placenta was already used as an efficient matrix for biomonitorization of metals (Hg), POPs and organo-chlorine pesticides in prenatal exposure (Vizcaino *et al*., 2014; Alves *et al*., 2017; Jeong *et al*., 2018). Some studies

suggest that is possible to determine newborn future diseases by analyzing the placenta (Andraweera *et al*., 2015).

The placental unit is composed by trophoblastic and fetal epithelial cells. The mature placenta develops different components that are characterized as chorionic plate, chorionic vili, decidua basalis and umbilical cord (Figure 1-5) (Cross *et al*., 2003).

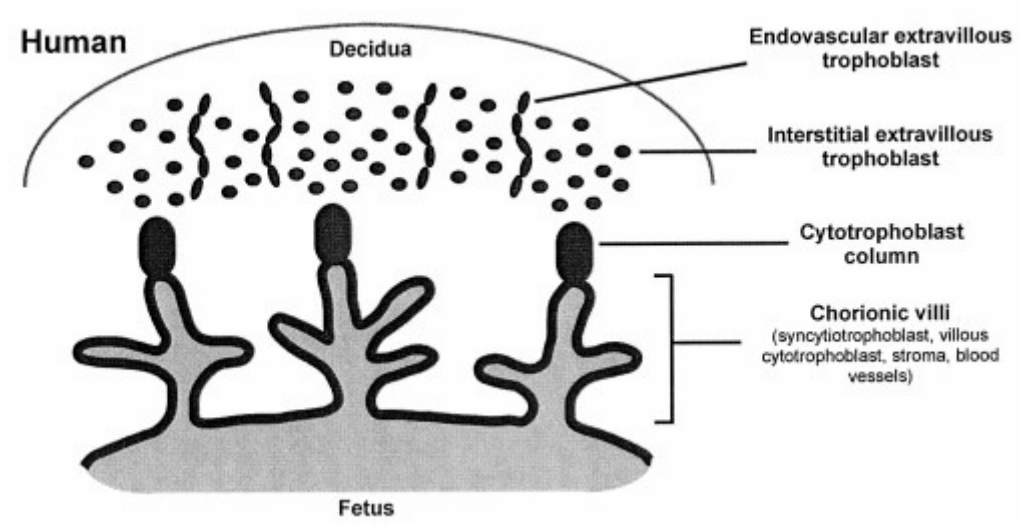

Figure 1-5 - Placenta representation (Cross *et al*., 2003).

Placenta is involved in fetal growth, development, nutrient transfer and products excretion thought the intervillous space. However, it is important to recognize other functionalities of placenta like metabolic and endocrine functions (Bauer *et al*., 1998). Xenobiotics can interfere with functions of placenta like signalling, hormonal effects, transport function, cell growth, maturation and delivery. Xenobiotics bioaccumulation can lead to preterm delivery, fetus weight alterations and in the worst case scenario malformations and fetus abortion (Myllynen, Pasanen and Pelkonen, 2005).

Nutrients' transfer and excretion between mother and fetus involves passive diffusion transfer of O_2 from the mother to the fetus and the excretion of CO_2 and urea from the fetus to the mothers' body. Facilitated diffusion mediates glucose and lactate transfer (Bauer *et al*., 1998). In this process, active transport with energy costs make amino acids pass through the membranes. In addition, pinocytosis, a transference mechanism based on the invagination of compounds (mostly drugs) through the membrane cell and aquaporins are water channel proteins that relocate water, mostly (Bauer *et al*., 1998; Barr, Bishop and Needham, 2007; Sha *et al*., 2011). Some toxins like nicotine and cocaine are transported like amino acids. In active transport,

exogenous compounds change placenta transfer of endogenous compounds. This interference with transporters can be adverse to fetal devolvement and damage in placenta functions (Myllynen, Pasanen and Pelkonen, 2005). Xenobiotics cross the placenta by simple passive diffusion or linked to proteins that pass through biological membranes. Xenobiotics enter to placenta through 20 different proteins, approximately. On the other side there are also some proteins that have the ability to prevent the entry of xenobiotics. The most well-known one is P-glicoprotein or the MDR1 gene product. P-glicoprotein has the ability to pump the substrates from intracellular to extracellular compartments. This protein was found in human placenta trophoblasts from first semester pregnancy to the baby delivery. It has a protection function in order to prevent teratogenic effects and fetal toxicity. Others like MRPs 1 to 5 (multi drugs resistance associated proteins) and BCRP (breast cancer resistance proteins) were found in the human mRNA from the first trimester pregnancy to the delivery (Myllynen, Pasanen and Pelkonen, 2005). Depending on the PAHs type, they enter the placenta through several processes like facilitated diffusion, passive diffusion, active transport and filtration (Sexton *et al*., 2011). Persistent compounds like PAHs can enter the hepatic blood stream circulation because of their lipophilic characteristic. They can continue in the fetal environment because they may be deposited and /or continue circulating in the hepatic blood stream. This way, through the umbilical cord and/or the umbilical cord blood, it is possible to understand if these compounds are in the fetal organism, so can be classified as a biomarker of exposure for the fetus too (Barr *et al*., 2007).

The pregnancy state is characterized by a higher oxidative stress in the mother and fetus. Placental mitochondrial activity induces oxidative stress, rise of ROS production (mainly the superoxide radical) and reduced scavenging function of antioxidants. The process of oxidation stress is characterized by an oxygen gradient between fetus and mother and it is affected by the higher ROS production in the mother circulation. ROS levels alterations during pregnancy state are important to assure normal growth of embryos and fetus (Myatt and Cui, 2004). The early post-implantation period is the most sensitive time to oxidative stress for the embryo. The uterus is hypoxic, in order to help the induction of proliferation of cells. Later on the fetus development the rise of oxidative stress can lead to differentiation and ROS act like a messenger for regulate transcription factors that could affect gene expression in embryo. During pregnancy the excessive increase of oxidative stress can be associated with miscarriage and other complications like pre-eclampsia, diabetes and intrauterine

growth retardation (Rossner Jr *et al*., 2009). One of the main functions of the placenta is promoting oxygen supply to the fetus. Lack of oxygen availability can lead to acute and chronic hypoxia (Macklin *et al*., 2017). Regulators of trophoblast, proliferation, and differentiation can lead to oxygen tension. Xenobiotics can damage regulation of cytotrophoblast, proliferation and differentiation can result in alterations of placental responses to oxygen tension (Myllynen, Pasanen and Pelkonen, 2005)

Once absorbed, PAHs can enter the lymph, circulate in all body through the blood and are primarily metabolized by the liver and kidneys (Kumar *et al*., 2014). They usually can bioaccumulate and be found in breast milk or adipose tissue because of is lipophilic characteristics and are excreted through urine or bile after being enzymatic metabolized into polar metabolites by responsible enzymes. In blood, PAHs have a half-life relatively short as it is a passage matrix. The PAHs concentrations in placenta usually are higher than PAHs concentrations in blood (Machado *et al*., 2014). Placenta is an organ that has the ability to bioaccumulate PAHs. At the end of the 1st trimester, the food intakes by the mother increases due to the higher levels of progesterone and hPL (human placental lactogen), while the metabolic demands are very low in mothers' body. This results in an increasing of fat that represents a loss of the normal homeostatic mechanism and regulate the energy balance (Burton and Fowden, 2015). The lipophilic characteristics of the fat perform as adsorption factor for PAHs species (Kumar *et al*., 2014). In placenta PAHs can be metabolized, being this organ referred often as an excretion route for PAHs due to the residence time of 9 months. There are other excretion routes in human body like milk, urine or feces (Kumar *et al*., 2014).

Biomarkers of exposure used in epidemiological studies are usually based on blood, urine or feces. There are positive and negative points in using placenta as a matrix for measurements of biomarkers of exposure. The collection and handling are the principal reasons to not use placenta as a matrix (Iyengar and Rapp, 2001), while the principal positive points are related to its big size, where one sample can be used to carry out several analytical measurements (chemical or biological) and it is considered a non-invasive matrix. Regarding PAHs, several compounds have been quantified in human placenta (Dejmek *et al*., 2000; Kampa and Castaño, 2009; Sexton *et al*., 2011; Yu *et al*., 2011; Chen *et al*., 2014; Machado *et al*., 2014; Tang *et al*., 2014; Yin *et al*., 2016; Urbancova *et al*., 2017).

1.8.2 Blood as a biomarker of PAHs exposure

In vertebrates, blood is a liquid conjunctive tissue formed by cells and plasma which circulates in a closed vascular system (capillary tubes). Blood cells can be categorized in red blood cells (RBC), white blood cells (WBC) and platelets. Blood main functions include transportation (nutrients, hormones, O_2 and CO_2), protection (leukocytes, antibodies and platelets) and regulation (pH and water balance). Blood circulates through all body, so it is possible to obtain general information on all tissues and organs. Several studies make use of samples of parturient and umbilical cord blood. The blood matrix is usually analyzed divided into plasma and blood cells or just as homogenized blood (Esteban and Castaño, 2009; Yu *et al*., 2011; Annamalai and Namasivayam, 2015).

EPA considers blood one of the best matrices for xenobiotics analyses (Madvan and Naidu, 1995). Blood extraction can be considered an invasive procedure, with some limitations considering the quantities required for analysis, being therefore needed to be collected in a way not to cause damage or considered harmful to humans (Esteban and Castaño, 2009). Blood can be used as a matrix to measure xenobiotic exposure from mother and baby. Significant concentrations of bisphenol A (BPA) were correlated with human umbilical cord blood. BPA concentrations can be related with some behavioral and mental problems in children (mostly until 42 months). Gender difference can be related with the alterations in the BPA metabolization (Minatoya *et al*., 2017). PFA (perfluoroalkyl substances) concentration measurements were collect from umbilical cord blood. The children were followed in this study until the 108 months and some correlations were detected with liver problems, thyroid problems, possible tumor formation, neural and growth problems. Different problems related with the children's gender can also occur (Chen *et al*., 2017).

Yu *et al*. (2011) performed PAHs measurement in placenta, umbilical cord blood and human milk. Umbilical cord blood presented the highest PAHs concentration of low molecular weight PAHs and the lowest concentration of high molecular weight PAHs. Madhavan and Naidu (1995) found a higher concentration of PAHs in umbilical cord compared with the maternal blood. This fact can be connected with the lipids and lipoproteins concentrations at higher quantities in the umbilical cord plasma (Sexton *et al*., 2011; Chen *et al*., 2014). The PAHs present in umbilical cord blood are correlated with cotinine concentrations and total PAHs levels were higher in smoker than in non-

smoker parturient. There were no significant differences between non-smoker parturient women and passive smoker parturient women (Machado *et al*., 2014). Most PAHs compounds have a half-life of 18 hour in the organism (Sexton *et al*., 2011; Machado *et al*., 2014).

1.9 Motivation, objectives and dissertation layout

Humans are exposed to chemical contamination through different sources, via inhalation, ingestion or dermic. These exposures are related directly or indirectly with environmental contamination but also with daily habits (e.g. smoking). The panoply of chemical compounds to which humans are exposed are enormous, going from pharmaceuticals, to metals, pesticides (e.g. organochlorides, carbamates), PAHs, amongst others (Kim *et al*., 2013).

In the present dissertation, PAHs were chosen as model chemicals to study human exposure. PAHs are persistent organic pollutants that have in their constitution hydrogen, carbon and benzene rings (Kim *et al*., 2013; Jeong *et al*., 2018). The number of benzene rings can fluctuate and originate different chemical species, varying from two (naphthalene equivalents) or three (phenanthrene equivalents) rings, and being classified as low molecular weight PAHs, to four (pyrene equivalents), five (BaP equivalents), six (Benzo[**ghi**]-perylene equivalents) or seven rings (indeno[1,2,3-**cd**] pyrene equivalents), classified as high molecular weight PAHs. They have some characteristics and different ways of adsorption in human body, which are associated with the number of benzene rings present (Kim *et al*., 2013). The identification, quantification and determination of PAHs in human samples are essential for an understanding of mechanisms, human health risk and PAHs related diseases (Figure 1-6) (Li *et al*., 2011).

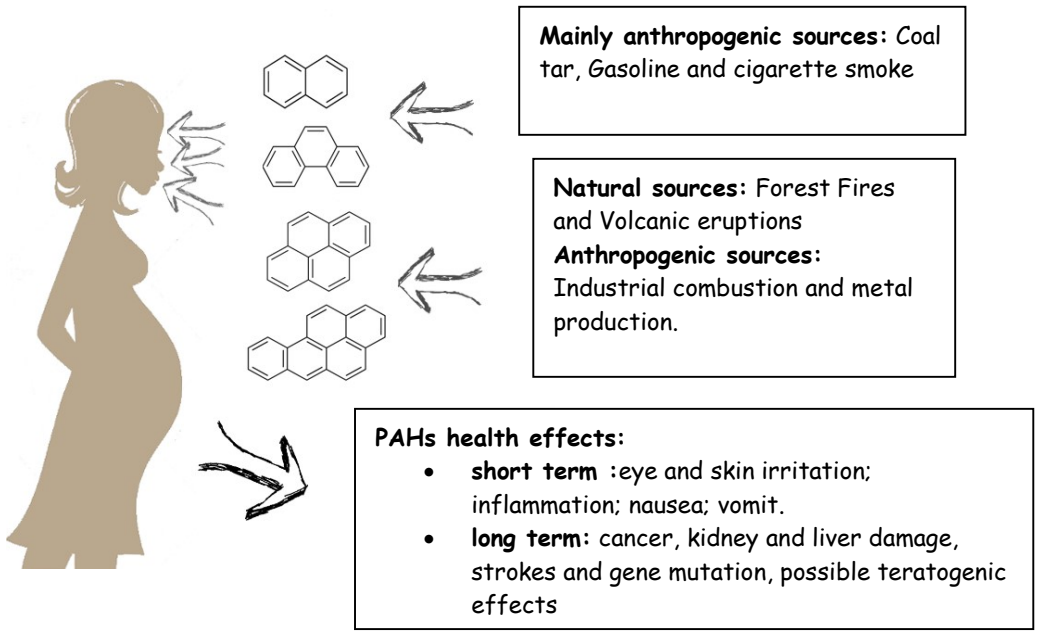

Figure 1-6 - LPAHs and HPAHs main sources and potential effects on human health (Adapted from Kim *et al*., 2013; Li *et al*., 2016).

There are several studies that measured PAHs levels in non-invasive human tissues (Sexton *et al*., 2011; Mohanraj, Dhanakumar and Solaraj, 2012; Tang *et al*., 2014; Yin *et al*., 2016). In Portugal, PAHs exposure has been quantified in food (Teixeira, Casal and Oliveira, 2007; Yebra-Pimentel *et al*., 2014), air (Slezakova *et al*., 2011; Albuquerque, Coutinho and Borrego, 2016) and sediments (Martins, Ferreira and Vale, 2008). Considering these exposure scenarios, some studies have already looked at PAHs concentrations in biological matrices as biomarkers of human exposure. The determination of PAHs levels in human urine (Oliveira *et al*., 2017) from 108 fire fighters from Vinhais, Mirandela and Bragança (north of Portugal) was carried out and urinary PAHs metabolites (OH-PAH) levels in firefighters that have been exposed to fire smoke were higher in those who smoke than in non-smoker firefighters (Oliveira *et al*., 2017).

In Aveiro region the principal PAHs source of emissions can be considered oil, industrial or domestics spills in Ria de Aveiro, vehicles emissions and burning smoke. Indication of exposure to these emissions are reported by the measurements of different PAHs species like naphthalene, acenaphthalene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[**a**]anthracene, chrysene, benzo[**b**]fluoranthene, benzo[**k**]fluoranthene, benzo[**a**]pyrene, benzo[**g,h,i**]perylene, dibenzo[**a,h**]anthracene and indeno[1,2,3- **c,d**]pyrene equivalents in eggs from different bird species in Aveiro region (Pegas *et al*., 2012) suggests an higher average values that

other bird species from Britain (Shore *et al*., 1999) or Neatherlands (Stronkhorst *et al*., 1993). These high PAHs concentrations can be still due to the Prestige incident, in 2010, or industrial or domestic spills in Ria de Aveiro (Vidal, Domínguez and Luís, 2011). In another study devoted to human exposure, between April and June of 2010, PM10 were measured inside and outside school buildings at suburban and center of Aveiro region. In Aveiro region, was found that suburban schools were exposed with more intensity to volatile organic compounds (VOCs) from industrial emissions than schools at the city center. The I/O ratios were higher in outdoor air than in indoors air space at schools, so it is possible that the main emission sources was vehicles smoke emission, biomass burning from restaurants and bakeries (Pegas *et al*., 2012).

The majority of PAHs measurements in human tissues rely on HPLC and mass spectrometer measurements, which may be considered expensive and time consuming. The development and use of other methodologies to increase rapidity and cost-efficiency can be promising as screening techniques (Li *et al*., 2011; Yu *et al*., 2011; Machado *et al*., 2014). Therefore methodologies based on PAHs properties like fluorescence have been developed, with the use of fluorimeters or a technique of fixed wavelength fluorescence. These methodologies can therefore provide a first screening insight on possible PAHs human exposure, helping stakeholders to act in a more efficient and prompt way, considering that PAHs exposure can lead to mutagenic, carcinogenic and teratogenic problems (Kim *et al*., 2013).

Main objectives

Considering the above, this study aims at determining potential human exposure to PAHs in the Aveiro region, Portugal, focusing on fetal exposure and relating maternal to fetus chemical transfer. For that, maternal and fetal matrices were collected (placenta, umbilical cord and mother blood) from parturient at the Infante D. Pedro Aveiro Hospital, Baixo Vouga Lagunar Medical Center and PAHs levels and metabolite types were quantified. PAHs levels were related to information gathered from questionnaires filled by parturient to infer on the influence of daily habits on PAHs exposure and accumulation, with special attention on information regarding smoke habits, industrial smoke and chemicals and motor combustion smoke exposure.

After this first chapter (Introduction), where state of the art on PAHs exposure and effects are described, this dissertation includes two additional chapters:

- Chapter II: "Polycyclic aromatic hydrocarbons levels in parturient and newborns from Aveiro region, Portugal". In this study, PAHs types were reported from placenta, umbilical cord blood and mothers' blood from 49 mothers/newborn pairs. Four PAHs species (naphthalene, phenanthrene, pyrene and BaP equivalents) were measured by a fixed wavelength fluorescence methodology in human placenta and blood (plasma and blood cells). The higher PAHs concentrations were found in homogenized placenta and were of naphthalene equivalents.
- Chapter III: In this chapter, there are a description of the major conclusions of the dissertation and perspectives of future studies.

1.10 References

Aas, E., Baussant, T., Balk, L., Liewenborg, B. and Andersen, O.K. 2000. "PAH Metabolites in Bile , Cytochrome P4501A and DNA Adducts as Environmental Risk Parameters for Chronic Oil Exposure : A Laboratory Experiment with Atlantic Cod." *Aquatic Toxicology* 51: 241–58. doi:10.1016/S0166-445X(00)00108-9.

Aas, E., Beyer, B., and Goksøyr, A. 2000. "Fixed Wavelength Fuorescence (FF) of Bile as a Monitoring Tool for Polyaromatic Hydrocarbon Exposure in Fish: An Evaluation of Compound Specificity, Inner Filter Effect and Signal Interpretation." *Biomarkers* 5 (1): 9–23. doi:10.1080/135475000230505.

Abdel-Shafy, H.I. and Ma N.nsour, M. 2015. "A Review on Polycyclic Aromatic Hydrocarbons: Source, Environmental Impact, Effect on Human Health and Remediation." *Egyptian Journal of Petroleum* 25 (1). Egyptian Petroleum Research Institute: 107–23. doi:10.1016/j.ejpe.2015.03.011.

Al-Rawashdeh, N. 2012. *Current Achievement and Future Potential of Fluorescence Spectroscopy, Macro To Nano Spectroscopy*.

Albuquerque, M., Coutinho, M. and Borrego, C. 2016. "Science of the Total Environment Long-Term Monitoring and Seasonal Analysis of Polycyclic Aromatic Hydrocarbons (PAHs) Measured over a Decade in the Ambient Air of Porto , Portugal." *Science of the Total Environment, The* 543. Elsevier B.V.: 439–48. doi:10.1016/j.scitotenv.2015.11.064.

Alves, A.C., Monteiro M.S., Machado, A.L., Oliveira, M., Bóia, A., Correia, A., Oliveira,N., Soares, A.M.V.M., Loureiro, S. 2017. "Mercury Levels in Parturient and Newborns from Aveiro Region , Portugal Mercury Levels in Parturient and Newborns from Aveiro Region , Portugal." *Journal of Toxicology and Environmental Health, Part A* 0 (0). Taylor & Francis: 1–13. doi:10.1080/15287394.2017.1286926.

Amazon, Barbecue. Available in: https://www.amazon.com/BBQ-Grill-Tools-Barbecue-Accessories/dp/B00ZTY7TMO: Access in: 22/08/2017

American Energy News, Gasoline. Available in: http://theamericanenergynews.com/energy-news/u-s-refiners-stuck-lots-gasoline-switch-winter-fuel; Access in : 22/08/2017

Andraweera, P H, Bobek, G., Bowen, C., Burton, G. J., Frigerio, P. C., Chaparro, A.,Dickinson, H., G. Duncombe, G., Hyett J., Illanes, S.E., Johnstone, E., Kumar, S., Morgan, T.K., Myers, J., Orefice, R., Roberts, C.T., Salafia, C.M., Thornburg, K.L., Whitehead, C.L., S.A. Bainbridge, S.A. 2015. "IFPA Meeting 2015 Workshop Report : Mechanistic Role of the Placenta in Fetal Programming ; Biomarkers of Placental Function and Complications of Pregnancy ; Late Onset Fetal Growth Restriction Surveillance and Monitoring." *Placenta*, 1–5. doi:10.1016/j.placenta.2015.12.012.

Annamalai, J., and Namasivayam, V. 2015. "Endocrine Disrupting Chemicals in the Atmosphere : Their Effects on Humans and Wildlife." *Environment International* 76. Elsevier Ltd: 78–97.

doi:10.1016/j.envint.2014.12.006.
Awebic, Colheita de sangue. Available in: https://awebic.com/saude/exame-de-sangue-pode-ser-o-futuro-no-diagnostico-de-cancer-de-mama/): Access in: 22/08/2017
Barr, D.B., Bishop,A. and Needham L. L.. 2007. "Concentrations of Xenobiotic Chemicals in the Maternal-Fetal Unit." *Reproductive Toxicology* 23: 260–66. doi:10.1016/j.reprotox.2007.03.003.
Bauer, M. K. , Harding J. E., Bassett N. S., Breier, B. H., Oliver, M. H., Gallaher, B. H., Evans, P. C., Woodall, S. M. and Gluckman, P. D. 1998. "Fetal Growth and Placental Function." *Molecular and Cellular Endocrinology* 140: 115–20.
Boving, T. B. and Zhang, W. 2004. "Removal of Aqueous-Phase Polynuclear Aromatic Hydrocarbons Using Aspen Wood Fibers." *Chemosphere* 54 (7): 831–39. doi:10.1016/j.chemosphere.2003.07.007.
Burton, G. J., and Fowden A. L. 2015. "The Placenta : A Multifaceted , Transient Organ." *Philosophical Transactions of the Royal Society B* 370. doi:10.1098/rstb.2014.0066.
Cduarouca wordpress, Forest fires. Available in: https://cduarouca.wordpress.com/2010/08/31/fogos-florestais-no-distrito-resultado-das-politicas-de-direita/: Access in: 22/08/2017
Chen, M.-H., Ng, S., Hsieh, C.-J., Lin, C.-C., Hsieh, W.-S. and Chen, P.-C. 2017. "Science of the Total Environment The Impact of Prenatal per Fluoroalkyl Substances Exposure on Neonatal and Child Growth." *Science of the Total Environment* 607–608. Elsevier B.V.: 669–75. doi:10.1016/j.scitotenv.2017.06.273.
Chen, Q., Zheng, T., Bassig, B., Cheng, Y., Leaderer, B., Lin, S., Holford, T., Qiu, J., Zhang, Y., Shi,K., Zhu, Y., Niu,J.,Li, Y., Guo, H., Hu, X., Jin, Y. 2014. "Prenatal Exposure to Polycyclic Aromatic Hydrocarbons and Birth Weight in China." *Open Journal of Air Pollution* 3 (December): 100–110. doi:10.4236/ojap.2014.34010.
Choi, H., Wang, L., Lin, X., Spengler, J. and Perera, F. P. 2012. "Fetal Window of Vulnerability to Airborne Polycyclic Aromatic Hydrocarbons on Proportional Intrauterine Growth Restriction." *PLoS ONE* 7 (4). doi:10.1371/journal.pone.0035464.
Clipartpanda, Blood eppendorf. Available in: http://www.clipartpanda.com/categories/centrifuge-20clipart; Access in: 22/08/2017
Clker, Blood eppendorf. Available in: http://www.clker.com/clipart-eppendorf-tube-red.html; Access in: 22/08/2017
Conti, G.O., Copat, C., Ledda, C., Fiore, M., Fallico R., Sciacca, S. and Ferrante, M. 2012. "Evaluation of Heavy Metals and Polycyclic Aromatic Hydrocarbons (PAHs) in Mullus Barbatus from Sicily Channel and Risk-Based Consumption Limits." *Bulletin of Environmental Contamination and Toxicology*, 946–50. doi:10.1007/s00128-012-0611-1.
Cross, J. C., Baczyk D., Dobric, N., Hemberger, M., Hughes, M., Simmons, D. G., Yamamoto H. and Kingdom,J. 2003. "Genes , Development and Evolution of the Placenta." *Placenta* 24: 123–30. doi:10.1053/plac.2002.0887.
Culturamix, Sangue do cordão umbilical. Available in: http://saude.culturamix.com/dicas/sangue-do-cordao-umbilical-caracteristicas-gerais: Access in: 22/08/2017
Decreto-Lei nº 306/2007. 2007. "Decreto-Lei N.º 306/2007." *Diário Da República: I Série*, no. 164: 5747–65.
Dejmek, J., Solanský, I., Benes, I., Lenícek J. and Srám R. J. 2000. "The Impact of Polycyclic Aromatic Hydrocarbons and Fine Particles on Pregnancy Outcome." *Environmental Health Perspectives* 108 (12): 1159–64. doi:sc271_5_1835 [pii].
Directive 2004/107/CE15 December 2004. 2005. *Official Journal of the European Union*.
Directive 2008/105/CE.2008. *Official Journal of the European Union*, 84–97.
Elken, Coal Tar. Available in: https://www.elkem.com/carbon/coal-tar-products/; Access in: 22/08/2017
Esteban, M., and Castaño, A. 2009. "Non-Invasive Matrices in Human Biomonitoring : A Review." *Environment International* 35 (2). Elsevier Ltd: 438–49. doi:10.1016/j.envint.2008.09.003.
Farhadian, A, Jinap, S., Faridah, A. and Zaidul, I.S. M. 2012. "Effects of Marinating on the Formation of Polycyclic Aromatic Hydrocarbons (Benzo [a] Pyrene , Benzo [B] Fl Uoranthene and Fl Uoranthene) in Grilled Beef Meat." *Food Control* 28 (2). Elsevier Ltd: 420–25. doi:10.1016/j.foodcont.2012.04.034.
Gámiz-Gracia, L., García-Campaña, A. M., Huertas-Pérez, J. F. and Lara, F. J. 2009. "Analytica Chimica Acta Chemiluminescence Detection in Liquid Chromatography : Applications to Clinical , Pharmaceutical , Environmental and Food Analysis — A Review" 640: 7–28. doi:10.1016/j.aca.2009.03.017.
Gladen, B. C., Zadorozhnaja, T. D., Chislovska, N., Hryhorczuk, D. O., Kennicutt M. C. and Little R. E. 2000. "Polycyclic Aromatic Hydrocarbons in Placenta," 597–603.
Glick, B. R. 2003. "Phytoremediation : Synergistic Use of Plants and Bacteria to Clean up the

Environment." *Biotechnology Advances 21* 21: 383–93. doi:10.1016/S0734-9750(03)00055-7.
Gravato, C. and Santos, M. A. 2003. "Genotoxicity Biomarkers' Association with B(a)P Biotransformation in Dicentrarchus Labrax L." *Ecotoxicology and Environmental Safety* 55 (3): 352–58. doi:10.1016/S0147-6513(02)00070-2.
Global Transport Knowledge Practice, Smoke transport. Available in : http://www.gtkp.com/themepage.php&themepgid=30; Access in : 22/08/2017
Healthy preschoolers, Meat, Milk and oil; Available in: https://www.healthypreschoolers .com/?page_id=542; Access in : 22/08/2017
Hites, R. A. 1997. "Gas Chromatography Mass Spectrometry." In *Handbook of Instrumental Techniques for Analytical Chemistry*, 609–26.
Hontela, A., Rasmussen, J. B., Audet, C., Chevalier, G. and Penfield, A. D. 1992. "Impaired Cortisol Stress Response in Fish from Environments Polluted by." *Archives of Environmental Contamination and Toxicology* 283: 278–83.
International Agency of Research on Cancer (IARC), 2010. Some Non heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 92. World Health Organization, Lyon, France, pp. 1e853
Iyengar, G V, and Rapp. A, 2001. "Human Placenta as a ' Dual ' Biomarker for Monitoring Fetal and Maternal Environment with Special Reference to Potentially Toxic Trace Elements . Part 3 : Toxic Trace Elements in Placenta and Placenta as a Biomarker for These Elements." *The Science of the Total Environment* 280: 221–38.
Jedrychowski, W., Perera, F., Tang , D., Rauh, V., Majewska R., Mroz E., Flak E. Stigter, L., Spengler, J. , Camann, D. and Jacek, R. 2013. "The relationship between prenatal exposure to airborne polycyclic aromatic hydrocarbons (pah) and pah-dna adducts in cord blood." *Journal of Exposure Science and Environmental Epidemiology* 23 (4): 371–77. doi:10.1038/jes.2012.117.THE.
Jeong, Y., Lee, S., Kim, S., Park, J., Kim, H.-J., Choi, G., Choi S., Kim S., Kim S.Y., Kim, S., Choi, K., Moon, H.-B. 2018. "Science of the Total Environment Placental Transfer of Persistent Organic Pollutants and Feasibility Using the Placenta as a Non-Invasive Biomonitoring Matrix." *Science of the Total Environment* 612. Elsevier B.V.: 1498–1505. doi:10.1016/j.scitotenv.2017.07.054.
Jung, K. H., Kim, J. K., Noh, J. H., Eun J. W., Bae H. J., Kim M. G., Chang, Y. G. Qingyu Shen a,b, Kim, S.-J., Kwon, S. H., Park, W. S., Jung Y. L., Nam, S. W. 2013. "Characteristic Molecular Signature for the Early Detection and Prediction of Polycyclic Aromatic Hydrocarbons in Rat Liver." *Toxicology Letters* 216 (1). Elsevier Ireland Ltd: 1–8. doi:10.1016/j.toxlet.2012.11.001.
Kampa, M. and Castanas, E. 2008. "Human Health Effects of Air Pollution." *Environmental Pollution* 151: 362–67. doi:10.1016/j.envpol.2007.06.012.
Kaupp, H. and Mclachlan, M.. 1999. "Atmospheric Particle Size Distributions of Polychlorinated Dibenzo- P -Dioxins and Dibenzofurans (PCDD / Fs) and Polycyclic Aromatic Hydrocarbons (PAHs) and Their Implications for Wet and Dry Deposition." *Atmospheric Environment* 33 (1): 85—95. doi:10.1016/S1352-2310(98)00129-0.
Kim, K.-H., Jahan, S. A., Kabir,E. and Brown, R. J. C. 2013. "A Review of Airborne Polycyclic Aromatic Hydrocarbons (PAHs) and Their Human Health Effects." *Environment International* 60. Elsevier Ltd: 71–80. doi:10.1016/j.envint.2013.07.019.
Kumar, S. N., Verma, P., Bastia,B. and Jain,A.K. 2014. "Health Risk Assessment of Polycyclic Aromatic Hydrocarbons: A Review." *Journal of Pathology and Toxicology* 1 (May 2016): 16–30.
Lai, I, Lee,C., Zeng,K. and Huang, H. 2011. "Seasonal Variation of Atmospheric Polycyclic Aromatic Hydrocarbons along the Kaohsiung Coast." *Journal of Environmental Management* 92 (8). Elsevier Ltd: 2029–37. doi:10.1016/j.jenvman.2011.03.026.
Li, X., Li,P., Yan,L., Chen,J., Chenga,T. and Xu, S. 2011. "Characterization of Polycyclic Aromatic Hydrocarbons in Fog–rain Events." *Journal of Environmental Monitoring* 13 (11). doi:10.1039/c1em10543d.
Li, Z., Wang, B., Ge, S., Yan, L., Liu, Y., Li, Z. and Ren, A. 2016. "A Simultaneous Analysis Method of Polycyclic Aromatic Hydrocarbons, Nicotine, Cotinine and Metals in Human Hair." *Environmental Pollution* 219. Elsevier Ltd: 66–71. doi:10.1016/j.envpol.2016.09.045.
Lin, E., Cormier, S. M. and Torsella, J. A. 1996. "Fish Biliary Polycyclic Aromatic Hydrocarbon Metabolites Estimated by Fixed-Wavelength Fluorescence : Comparison." *Ecotoxicology and Environmental Safety* 23 (35): 16–23. doi:10.1006/eesa.1996.0077.
Lingeman, H, Underberg, W. J. M., Takedate, A., and Hulshoff, A. 1985. "Fluorescence Detection in High Performance Liquid Chromatography." *Journal of Liquid Chromatography*, no. June 2013: 37–41. doi:10.1080/01483918508067120.
Machado, J., Chatkin, J. M., Zimmer, A. R., Goulart A. P., and Thiesen, F. 2014. "Cotinine and

Polycyclic Aromatic Hydrocarbons Levels in the Amniotic Fluid and Fetal Cord at Birth and in the Urine from Pregnant Smokers." *PLoS ONE* 9 (12): 1–12. doi:10.1371/journal.pone.0116293.

Macklin, P. S, Mcauliffe, J., Pugh, C.W. and Yamamoto, A. 2017. "Hypoxia and HIF Pathway in Cancer and the Placenta." *Placenta*. Elsevier Ltd, 1–6. doi:10.1016/j.placenta.2017.03.010.

Madhavan, N. D. and Naidu, K. A. 1995. "Polycyclic Aromatic Hydrocarbons in Placenta , Maternal Blood , Umbilical Cord." *Human & Experimental Toxicology*, no. 14: 503–6. doi:10.1177/096032719501400607.

Mariajoaoalmeida, Fruit. Available in: http://mariajoaodealmeida.com/?p=4628; Access in: 22/08/2017

Martins, M., Ferreira,A. and Vale, C. 2008. "The Influence of Sarcocornia Fruticosa on Retention of PAHs in Salt Marsh Sediments (Sado Estuary, Portugal)." *Chemosphere* 71 (8): 1599–1606. doi:10.1016/j.chemosphere.2007.10.054.

Martorell, I., Perelló, G., Martí-Cid, R., Castell, V., Llobet,J. and Domingo, J. 2010. "Polycyclic Aromatic Hydrocarbons (PAH) in Foods and Estimated PAH Intake by the Population of Catalonia, Spain: Temporal Trend." *Environment International* 36 (5). Elsevier Ltd: 424–32. doi:10.1016/j.envint.2010.03.003.

Meire, R. O., Azeredo, A. and Torres, J. P. 2007. "AROMÁTICOS." *Oecologia Brasiliensis* 11 (2): 188–201.

Minatoya, M., Araki, A., Nakajima,S., Sasaki,S., Miyashita,C. and Yamazaki, K. 2017. "Science of the Total Environment Cord Blood BPA Level and Child Neurodevelopment and Behavioral Problems : The Hokkaido Study on Environment and Children â€™ S Health." *Science of the Total Environment* 607–608. Elsevier B.V.: 351–56. doi:10.1016/j.scitotenv.2017.06.060.

Mohanraj, R., Dhanakumar, S.and Solaraj, G. 2012. "The Cientific WorldJOURNAL Polycyclic Aromatic Hydrocarbons Bound to PM 2 . 5 in Urban Coimbatore , India with Emphasis on Source Apportionment." *The ScientificWorld Journal* 2012: 8. doi:10.1100/2012/980843.

Mundo educação, Vulcão. Available in: http://mundoeducacao.bol.uol.com.br/geografia/10-curiosidades-sobre-vulcoes.htm; Acess in: 22/08/201

Myatt, L. and Cui, X. Histochem Cell Biol (2004) 122: 369. https://doi.org/10.1007/s00418-004-0677-x

Myllynen, P., Pasanen M., and Pelkonen, O. 2005. "Human Placenta : A Human Organ for Developmental Toxicology Research and Biomonitoring." *Placenta*, no. 26: 361–71. doi:10.1016/j.placenta.2004.09.006.

Oliva, M., González de Canales, M. L., Gravato, C., Guilhermino, L., and Perales, J. A. 2010. "Biochemical Effects and Polycyclic Aromatic Hydrocarbons (PAHs) in Senegal Sole (Solea Senegalensis) from a Huelva Estuary (SW Spain)." *Ecotoxicology and Environmental Safety* 73 (8): 1842–51. doi:10.1016/j.ecoenv.2010.08.035.

Oliveira, M., Slezakova, K., Magalhães C., Fernandes, A., Teixeira, J. P., Delerue-Matosa, C., Pereira,M. and Morais, S. 2017. "Individual and Cumulative Impacts of Fire Emissions and Tobacco Consumption on Wildland Firefighters' Total Exposure to Polycyclic Aromatic Hydrocarbons." *Journal of Hazardous Materials*. Elsevier B.V. doi:10.1016/j.jhazmat.2017.03.057.

Organizar a casa, Peixe. Available in: http://www.organizaracasa.com/cozinhar-peixe/; Access in: 22/08/2017

Pegas, P. N., Nunes, T. Alves, C. A., Silva, J. R., Vieira, S. L. A., Caseiro, A. and Pio, C. A. 2012. "Indoor and Outdoor Characterisation of Organic and Inorganic Compounds in City Centre and Suburban Elementary Schools of Aveiro , Portugal." *Atmospheric Environment* 55. Elsevier Ltd: 80–89. doi:10.1016/j.atmosenv.2012.03.059.

Perera, F., Tang W., Herbstman, J., Tang, D., Levin, L. and Miller, R. 2009. "Relation of DNA Methylation of 5 9 -CpG Island of ACSL3 to Transplacental Exposure to Airborne Polycyclic Aromatic Hydrocarbons and Childhood Asthma" 4 (2). doi:10.1371/journal.pone.0004488.

Polanska, K., Hanke, W., Sobala, W. and Brzeźnicki, S. 2010. "Exposure to Polycyclic Aromatic Hydrocarbons and newborn" 23 (4): 339–46. doi:10.2478/v10001-010-0028-1.

Preuss, R., Angerer, J. and Drexler, H.. 2003. "Naphthalene - An Environmental and Occupational Toxicant." *International Archives of Occupational and Environmental Health* 76 (8): 556–76. doi:10.1007/s00420-003-0458-1.

Ren, A., Qiu, X., Jin, L., Ma, J., Li, Z., Zhang, L., Zhu, H., and Finnell, R. H. 2011. "Association of Selected Persistent Organic Pollutants in the Placenta with the Risk of Neural Tube Defects." *Environmental Sciences* 108 (31): 12770–75. doi:10.1073/pnas.1105209108.

Rossner Jr, P., Milcova, A., Libalova H., Novakova Z., Topinka J., Balascak I., and Sram, R. J. 2009. "Mutation Research / Fundamental and Molecular Mechanisms of Mutagenesis Biomarkers of Exposure to Tobacco Smoke and Environmental Pollutants in Mothers and Their Transplacental Transfer to the Foetus . Part II . Oxidative Damage." *Mutation Research* 669: 20–26. doi:10.1016/j.mrfmmm.2009.04.010.

Scheererbearing, Metal production. Available in: http://www.scheererbearing.com/industries/steel-metals/; Access in: 22/08/2017

Sexton, K., Salinas, J. J., McDonald, T.J., Gowen, R.M. Z., Miller, R. P., McCormick, J.B., and Fisher-Hoch, S.P.. 2011. "Polycyclic Aromatic Hydrocarbons in Maternal and Umbilical Cord Blood from Pregnant Hispanic Women Living in Brownsville, Texas." *International Journal of Environmental Research and Public Health* 8 (8): 3365–79. doi:10.3390/ijerph8083365.

Sha, X., Xiong, Z., Liu, H., Di, X. and Ma, T. 2011. "Maternal-Fetal Fluid Balance and Aquaporins : From Molecule to Physiology." *Acta Pharmacologica Sinica* 32 (6). Nature Publishing Group: 716–20. doi:10.1038/aps.2011.59.

Shore, R. F., Wright, J., Horne, J. A. and Sparks, T. H. (1999). Polycyclic aromatic hydrocarbon (PAH) residues in the eggs of coastal-nesting birds from Britain. *Marine Pollution Bulletin*, *38*(6), 509-513.

Sigma-Aldrich, Benzo[**a**]pyrene; Available in: http://www.sigmaaldrich.com/catalog/substance/benzoapyrene252315032811?lang=pt&region=PT; Access in: 22/08/2017a

Sigma-Aldrich, Naphthalene. Available in: http://www.sigmaaldrich.com/catalog/product/aldrich/147141?lang=pt&region=PT; Access in: 22/08/2017b

Sigma-Aldrich, Phenanthrene. Available in: http://www.sigmaaldrich.com/catalog/substance/phenanthrene178238501811?lang=pt&region=PT; Access in: 22/08/2017c

Sigma-Aldrich, Pyrene. Available in: http://www.sigmaaldrich.com/catalog/substance/pyrene2022512900011?lang=pt&region=PT; Access in: 22/08/2017d

Singh, V. K., Patel D. K., Ram, S., Mathur, N. and Siddiqui M. K. J. 2008. "Blood Levels of Polycyclic Aromatic Hydrocarbons in Children and Their Association with Oxidative Stress Indices : An Indian Perspective." *Archives of Environmental Contamination and Toxicology* 41 (54): 152–61. doi:10.1016/j.clinbiochem.2007.11.017.

Skillplus, Industrial smoke. Available in: https://skillplus.wordpress.com/2012/04/23/industrial-smoke-brushes-for-photoshop/; Access in:22/008/2017

Slezakova, K., Castro, D., Begonha, A., Delerue-Matos C., Alvim-Ferraz, C., Morais, S. and Pereira,C. 2011. "Air Pollution from Traffic Emissions in Oporto , Portugal : Health and Environmental Implications." *Microchemical Journal* 99 (1). Elsevier B.V.: 51–59. doi:10.1016/j.microc.2011.03.010.

Smith, J. A., and Galan A. 1995. "Sorption of Nonionic Organic Contaminants to Single and Dual Organic Cation Bentonites from Water." *Environmental Science and Technology* 29 (3): 685–92. doi:10.1021/es00003a016.

Srogi, K. 2007. "Monitoring of Environmental Exposure to Polycyclic Aromatic Hydrocarbons: A Review." *Environmental Chemistry Letters* 5 (4): 169–95. doi:10.1007/s10311-007-0095-0.

Stronkhorst, J., Ysebaert, T. J., Smedes, F., Meininger, P. L., Dirksen, S., and Boudewijn, T. J. 1993. "Contaminants in Eggs of Some Waterbird Species from the Scheldt Estuary, SW Netherlands." *Marine Pollution Bulletin* 26 (10): 572–78. doi:10.1016/0025-326X(93)90409-D.

Sudakin, D., Stone,D. and Power, L.. 2011. "Their Relevance to Environmental Health." *Current Topics in Toxicology* 9385 (541): 13–19. doi:10.1016/j.scitotenv.2015.05.123.

Tang, D., Li, T., Chow, J., Kulkarni, S., Watson, J., Ho,S., Quan, Z., Qu, L. and Perera, F. 2014. "Air Pollution Effects on Fetal and Child Development : A Cohort Comparison in China." *Environmental Pollution* 185: 90–96. doi:10.1016/j.envpol.2013.10.019.

Teixeira, V. H., Casal, S. and Oliveira, B. 2007. "Food Chemistry PAHs Content in Sunflower , Soybean and Virgin Olive Oils : Evaluation in Commercial Samples and during Refining Process" 104: 106–12. doi:10.1016/j.foodchem.2006.11.007.

Time, Smoke. Available in: http://healthland.time.com/2012/09/21/the-major-toll-of-secondhand-smoke/; Access in 22/08/2017

UE Directive 2015/1787 of 2015 October.2015. *Official Journal of the European Union* 2015 (4): 27–29.

Urbancova, K., Lankova,D., Rossner,P., Rossnerova,A., Svecova,V., Tomaniova,M., Veleminsky,M., Sram, R.J., Hajslova,J., and Pulkrabova,J. 2017. "Evaluation of 11 Polycyclic Aromatic Hydrocarbon Metabolites in Urine of Czech Mothers and Newborns." *Science of the Total Environment* 577: 212–19. doi:10.1016/j.scitotenv.2016.10.165.

Vidal, M., Domínguez,J. and Luís, A. 2011. "Science of the Total Environment Spatial and Temporal Patterns of Polycyclic Aromatic Hydrocarbons (PAHs) in Eggs of a Coastal Bird from Northwestern Iberia after a Major Oil Spill." *Science of the Total Environment, The* 409 (13). Elsevier B.V.: 2668–73. doi:10.1016/j.scitotenv.2011.03.025.

Vizcaino, E., Grimalt,J., Fernández-Somoano,A. and Tardon, A. 2014. “Transport of Persistent Organic Pollutants across the Human Placenta.” *Environment International* 65. Elsevier Ltd: 107–15. doi:10.1016/j.envint.2014.01.004.

Vo-Dinh, T. 1978. “Multicomponent Analysis by Synchronous.” *ANALYTICAL CHEMISTRY* 50 (3): 396–401. doi:10.1021/ac50025a010.

Wang, Z., Ren, P., Sun, Y., Ma, X., Liu, X., Na, G., and Yao, Z. 2013. “Gas/particle Partitioning of Polycyclic Aromatic Hydrocarbons in Coastal Atmosphere of the North Yellow Sea, China.” *Environmental Science and Pollution Research* 20 (8): 5753–63. doi:10.1007/s11356-013-1588-y.

Wefer-Roehl, A., Graber, E. R., Borisover, M. D., Adar, E., Nativ, R.and Ronen, Z. 2001. “Sorption of Organic Contaminants in a Fractured Chalk Formation.” *Chemosphere* 44 (5): 1121–30. doi:10.1016/S0045-6535(00)00309-X.

Wixstatic, Naphthalene balls. Available in: http://static.wixstatic.com/media/85ccb6_cd5ff7775724c407c768a450f752912c.png_srz_900_410_85_22_0.50_1.20_0.00_png_srz; Access in: 22/08/2017

Wilson, S. C., and Jones, K. C.. 1993. “BIOREMEDIATION OF SOIL CONTAMINATED WITH POLYNUCLEAR AROMATIC HYDROCARBONS (PAHs): A REVIEW.” *Environmental Pollution* 81: 229–49. doi:0269-7491/93/$06.0.

World Health Organization, WHO. (2010). WHO guidelines for indoor air quality: selected pollutants

Yebra-Pimentel, I., Fernández-González, R., Martínez-Carballo E., and Simal-Gándara, J. 2014. “Critical Reviews in Food Science and Nutrition A Critical Review about the Health Risk Assessment of PAHs and Their Metabolites in Foods.” *Critical Reviews in Food Science and Nutrition*, 37–41. doi:10.1080/10408398.2012.697497.

Yin, S., Tang, M., Chen, F., Li, T. and Liu, W. 2016. “Environmental Exposure to Polycyclic Aromatic Hydrocarbons (PAHs): The Correlation with and Impact on Reproductive Hormones in Umbilical Cord Serum.” *Environmental Pollution*, 1–9. doi:10.1016/j.envpol.2016.10.090.

Yu, Y., Wang X., Wang, B., Tao S., Liu W., Wang X., Cao, J., Li B., Lu, X., and Wong, M.H. 2011. “Polycyclic Aromatic Hydrocarbon Residues in Human Milk, Placenta, and Umbilical Cord Blood in Beijing, China.” *Environmental Science & Technology* 45 (23): 10235–42. doi:10.1021/es202827g.

Zeledón-Toruño, Z. C., Lao-Luque, C., Las Heras, F. and Sole-Sardans, M. 2007. “Removal of PAHs from Water Using an Immature Coal (Leonardite).” *Chemosphere* 67 (3): 505–12. doi:10.1016/j.chemosphere.2006.09.047.

Zhang, S., Zhang, W., and Wang, K. 2009. “Concentration , Distribution and Source Apportionment of Atmospheric Polycyclic Aromatic Hydrocarbons in the Southeast Suburb of Beijing , China.” *Environmental Monitoring and Assessment*, 197–207. doi:10.1007/s10661-008-0261-2.

Chapter II

Polycyclic aromatic hydrocarbons levels in parturient and newborns from Aveiro region, Portugal

Fraga, M[1]; Gravato, C[2]; Monteiro, MS[1]; Silva, C[1]; Machado, AL[1]; Soares, AMVM[1]; Alves, AC[3]; Loureiro, S[1]

[1]Departamento de Biologia & CESAM, Universidade de Aveiro, 3810-193 Aveiro, Portugal
[2]Faculdade de Ciências & CESAM, Universidade de Lisboa, 1749-016 Lisboa, Portugal
[3]I3S, Porto, Portugal

Abstract

Humans can be exposed to different PAHs since they are considered ubiquitous environmental contaminants that arise from several natural and anthropogenic sources. PAHs are considered a group of persistent priority chemicals that present carcinogenic and teratogenic effects on organisms. Therefore, it is crucial to monitor and study human exposure to PAHs, namely during fetal development, which is considered a sensitive window of exposure to contaminants. The main goals of this study were: (i) to assess maternal and fetal exposure to PAHs in parturient from the Aveiro region using placenta and blood as biological matrices; (ii) to examine the influence of environmental, sociodemographic, lifestyle and smoking habits that may contribute to PAHs exposure during pregnancy in Aveiro region, Portugal. For this, a fast screening tool based on the inherent fluorescence properties of PAHs (dependent on their number of benzene rings) was used to quantify the levels of 2-, 3-, 4- and 5-ring PAHs equivalents in human biological matrices.

In this study, 49 mother/newborn pairs from the Aveiro region were used after their agreement and ethical consent. Parturient filled a questionnaire on their daily habits and personal details and biological samples were collected, namely, placenta, umbilical cord blood and mothers' blood. Fixed fluorescence wavelength was used to quantify levels of PAHs in the tissues collected after calibration and determination of the techniques LOD and LOQ for each standard compound used using the respective exc/em wavelength pair.

In general, within the matrices studied, the studied group presented higher levels of PAHs equivalents in placenta (total homogenate fraction) and lower levels in the umbilical cord blood. Low molecular weight PAHs (naphthalene and phenanthrene) measured in placenta presented higher levels than high molecular weight PAHs (pyrene

and benzo[**a**]pyrene). Considering the county of residence, the highest PAHs levels in placenta were found in parturient from Aveiro, Ílhavo and Albergaria-a-Velha and the lowest levels were observed in parturient from Águeda. Moreover, increased levels of naphthalene and phenanthrene equivalents were associated with mothers' exposure to vehicle exhaust, while high levels of benzo[**a**]pyrene equivalents were associated with their exposure to tobacco smoke at work. The highest levels of naphthalene, phenanthrene and BaP equivalents were found in homogenized placenta of mothers who smoked during the third trimester of pregnancy. No significant correlations were found between levels of PAHs equivalents in biological matrices studied and anthropometric measures of newborns, but in general, high levels of PAHs equivalents were found in newborns that presented low body weight and length, as well as, smaller head circumference.

Biomonitorization of newborns and parturient can be a major asset in evaluating environmental exposure to contaminants, which can also provide high value information for preventive medicine. A different method was adapted for detection of low- and high molecular PAHs equivalents in tissues seems to be a fast and sensitive screening biomarker tool that allows analyses of large quantities of samples.

Keywords: Polycyclic aromatic hydrocarbons, pre-natal exposure, biomonitoring, biomarkers of exposure, human blood, human placenta.

2.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of persistent organic compounds constituted by fused or condensed aromatic benzene ring structures. The number of benzene ring structures can vary between 2 and 7, which is the basis for their classification as low molecular weight (LPAHs; 2- and 3-benzene rings) and high molecular weight PAHs (HPAHs; above 4-benzene rings) (Kim *et al*., 2013). PAHs can be transported in human matrices (e.g. blood) depending on the physic-chemical properties such as water solubility, hydrophobicity, molecular weight and size. Low molecular weight PAHs have a smaller size, are found in volatile phase and have a smaller Kow, mainly. High molecular weight PAHs have a bigger size, found adsorbed in particulate phase and have larger Kow (Kim *et al*., 2013; Jeong *et al*., 2018). Thus, naphthalene (with two benzene rings; log Kow =3.37) and phenanthrene (three rings; log Kow= 4.57) are examples of LPAHs, whereas, pyrene (four rings; log Kow = 4.88) and benzo[**a**]pyrene (BaP; five rings; log Kow= 6.04) are examples of HPAHs (Annamalai and Namasivayam, 2015; Abdel-Shafy and Mansour, 2015). The PAHs species with a larger Kow (log Kow > 2) tend to accumulate in tissues (Stronkhorst et *al*., 1993).

In the environment, PAHs can occur in vapour phase or bound to airborne particles that might be associated also with other pollutants. However, PAHs phase depends on the atmospheric conditions, their properties and origin. Moreover, PAHs are also present in soil, water and food due to deposition (Kim *et al*., 2013). Most of the anthropogenic sources arise from the combustion of organic materials. However, there are other anthropogenic sources like the incomplete burn of garbage, tobacco and fuel. Forest fires and volcanic eruptions are considered natural sources of PAHs (Kim *et al*., 2013; Urbancova *et al*., 2017)

Therefore, humans are frequently exposed to these persistent organic pollutants through natural and/or anthropogenic sources. Human exposure to PAHs can occur through inhalation, ingestion or skin contact (Kim *et al*., 2013) and bioaccumulation in tissues and deleterious effects are expected to occur depending on their physico-chemical properties, exposure route and origin (Sexton *et al*., 2011; Kim *et al*., 2013; Chen *et al*., 2014; Machado *et al*., 2014; Yin *et al*., 2016; Urbancova *et al*., 2017).

Human health effects are related to short (eye and skin irritation; inflammation; nausea; vomit) (Kim *et al*., 2013) and long term exposures, which may cause endocrine

disruption (Yin *et al*., 2016) mutagenic, teratogenic and carcinogenic problems (IARC, 2010; Kim *et al*., 2013; Chen *et al*., 2014), fetal development toxicity and repercussions in human reproduction (Yu *et al*., 2011), risk of cell damage and cardiopulmonary problems (Kuo *et al*., 2003), and ultimately death.

Epidemiological studies require biomarkers to quantify the individual exposure, population susceptibility and health effects (Suk, Collman and Damstra, 2014). Urine and blood are the more common used and both considered good biological matrices for human biomonitoring. Blood is known to have a lower potential to accumulate PAHs than other tissues and/or organs (Pleil *et al*., 2010), since these compounds tend to accumulate in more lipophilic tissues or organs due to their lipophilic nature. However, a previous study showed that high levels of PAHs were accumulated in maternal blood (Sexton *et al*., 2011). Placenta is also referred as a useful matrix when aiming at evaluates both maternal and fetal exposure to contaminants, mother to fetal transference, development toxicity and newborns health status. This is a large and discoid organ whose main functions are related to fetal growth and development, nutrient and excretion transference and has also metabolic and endocrine functions (Bauer *et al*., 1998; Machado *et al*., 2014). The placenta has been used in epidemiological and biomonitoring studies since 1974 (Baglan *et al*., 1974), as a non-invasive matrix that provides relevant information about xenobiotics bioaccumulation. In the study of Yu *et al*. (2011) and Sexton *et al*. (2011) the accumulation of different PAHs **(**naphthalene, acenaphthylene, acenaphthene, fluorine, phenanthrene, anthracene, fluoranthene, pyrene, benz[**a**]anthracene, chrysene, benzo[**b**]fluoranthene, benzo[**k**]fluoranthene, benzo[**a**]pyrene, dibenz[**a**,**h**]anthracene, indeno[l,2,3-**cd**]pyrene and benzo[**g**,**h**,**i**]perylene) were reported in human placenta. Moreover, Machado *et al*. (2014) found that PAHs can be transmitted from mother to fetus blood through placenta. Other studies found significant correlations between levels of low molecular weight (LPAHs) in the placenta, human milk and umbilical cord blood also suggesting their transfer from the mother to the fetus (Yu *et al*., 2011).

Although it is commonly known that PAHs exposure can be harmful in adulthood, pre-natal and childhood exposure can lead to a higher risk since it represents a sensitive window of human development where a smaller body mass has to cope already in an early stage with contaminant load (Tang *et al*., 2014).

In this context, the present study has the following main goals: (i) to assess maternal and fetal exposure to PAHs in parturient from the Aveiro region using placenta and

blood as biological matrices, and (ii) to examine the influence of environmental, sociodemographic, lifestyle and smoking habits that may contribute to PAHs exposure during pregnancy in Aveiro region, Portugal. Moreover, it was intended to adapt an already described fast screening method described by Gravato and Santos (2003) and Oliva *et al*. (2010) to quantify PAHs equivalent levels in human matrices. The methodology was adjusted to microplates and can represent a useful and cheap tool based on PAHs fluorescence properties as function of ring numbers.

2. 2. Materials and Methods

2.2.1 Study Design, subjects and Sampling

This work consisted in a cross-sectional study performed in the same individuals reported by Alves *et al*. (2017). Briefly, 49 parturient women resident in Aveiro region were randomly selected for the study from October 2014 to April 2015 at Infante D. Pedro Hospital (Centro Hospitalar do Baixo Vouga, Aveiro, Portugal), during their delivery. Aveiro is a district located in the central-north Atlantic coast in mainland of Portugal, and women involved in the study had their residence in the following counties: Águeda; Albergaria-A-Velha; Aveiro; Estarreja; Ílhavo; Oliveira do Bairro; Ovar; Vagos; Sever do Vouga; Anadia.

All parturient were interviewed before or within the first 24 hours after delivery using a standardized questionnaire to collect sociodemographic data, e.g. maternal age (years); body mass index (BMI) before pregnancy and weight gain during pregnancy (kg); time of residence (years); place of residence (urban or rural; next to roads with high traffic); maternal education (university graduated; high school graduated; primary graduated or attend some years in primary school); parity (0,+1); occupational exposure (work status -unemployed or employed-), exposure to chemicals (yes or no); smoking habits (yes or no or passive smoker and how many cigarettes /day), anthropometric data of the newborn (head circumference; birth length; birth weight). All parturient participating in this study were informed and signed the consent form. Furthermore, this study was approved by the Ethics Committee of Infante D. Pedro Hospital in Centro Hospitalar do Baixo Vouga, Aveiro.

Regarding the methods and procedures, placenta and blood samples from parturient and umbilical cord were collected and stored at -80ºC until further analysis. The placenta tissue was defrosted on ice and homogenized with phosphate buffer 0.1M pH 7.4 (100 mg tissue per 1 mL buffer) using an ultrasonic cell disruption procedure (Branson 250 sonifier). Part of the homogenized placenta was centrifuged (10.000 g; 20 min; 4ºC) to obtain the supernatant (soluble part of the cells, the cytosol). Both homogenized placenta and supernatant fractions were stored in -80ºC freezer until further analysis. Blood samples from parturient and umbilical cord were collected and stored in anticoagulant and the plasma was separated from blood cells in the same day of collection through centrifugation (500 g, 5 min, 4ºC). The pure plasma was stored in -80ºC freezer. The blood cells pellets were ressuspended in phosphate buffer 0.1M pH 7.4 and then stored at -80ºC.

2.2.2 Quantification of protein content

The method of Bradford (1976) adapted to microplate was used for protein quantification in all the samples of placenta, plasma and blood cells using a wavelength of 600 nm (Lab system Multiskan EX microplate reader) and gamma-globulin as standard. Protein content was quantified in order to be used in the normalization of levels of PAHs present in these tissues.

2.2.3 Analytical procedure for PAHs

A fluorescence spectrophotometer (HITACHI F-7000) was used to quantify PAHs levels in the sampled tissues. Four types of PAHs were determined according to their number of rings using naphthalene (2-rings), phenanthrene (3-rings), pyrene (4-rings) and benzo[**a**]pyrene (5-rings) as standards. Fluorescence analysis was performed in accordance with the method described by Oliva *et al*. (2010), where naphthalene-type PAHs or equivalents were measured at 290/335 nm (excitation/emission pair) (Figure 2-1), phenanthrene-type PAHs or equivalents at 259/380 nm (Figure 2-2), pyrene-type PAHs or equivalents at 341/383 nm (Figure 2-3) and benzo[**a**]pyrene-type PAHs or equivalents at 380/430 nm (Figure 2-4). Each standard curve was performed 4

times and each standard was measured in 4 replicates at each time a curve was performed.

Then, equivalents of these 4 PAHs were quantified in mothers' blood cells and blood plasma, umbilical' cord cells and plasma, as well as, homogenized samples and S9 fraction or supernatant of placenta. Concisely, plasma, blood cells and placenta samples were defrosted at room temperature (25ºC) and were further diluted in methanol 50% in a 1:30 proportion. Then, microtubes were sonicated (P Electra ultrasonic) for 1 minute and 4 technical replicates per sample were used in a 96-well microplate to quantify levels of PAHs equivalents according to their number of benzene rings.

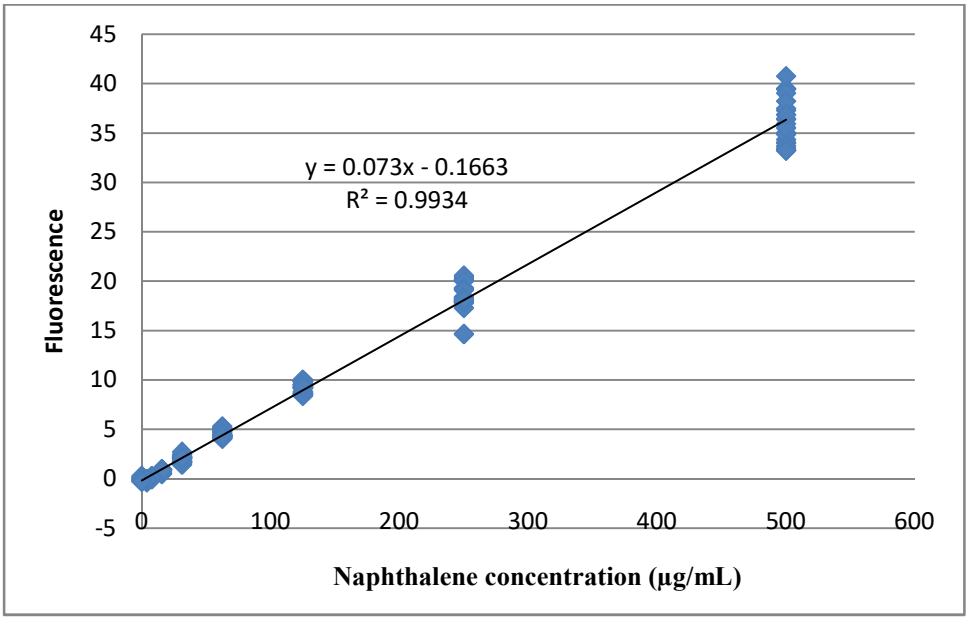

Figure 2-1 - Fluorescence intensity using a range of concentrations of 3.91 until 125 µg/mL naphthalene. Each concentration of naphthalene was determined 4 times and for each determination 4 replicates were done, including a control, with no contaminant added. Linear adjustment (R^2) and equation are shown in the figure. Linear equation was used for the calculation of naphthalene equivalents in samples.

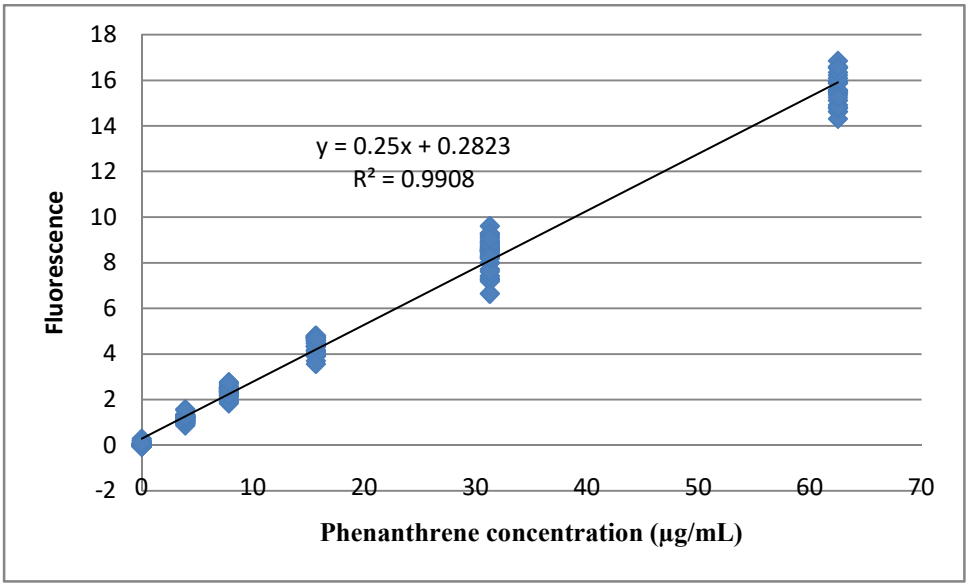

Figure 2-2 - Fluorescence intensity using a range of concentrations of 3.91 until 62.5 µg/mL phenanthrene. Each concentration of phenanthrene was determined 4 times and for each determination 4 replicates were done, including a control, with no contaminant added. Linear adjustment (R^2) and equation are shown in the figure. Linear equation was used for the calculation of naphthalene equivalents in samples.

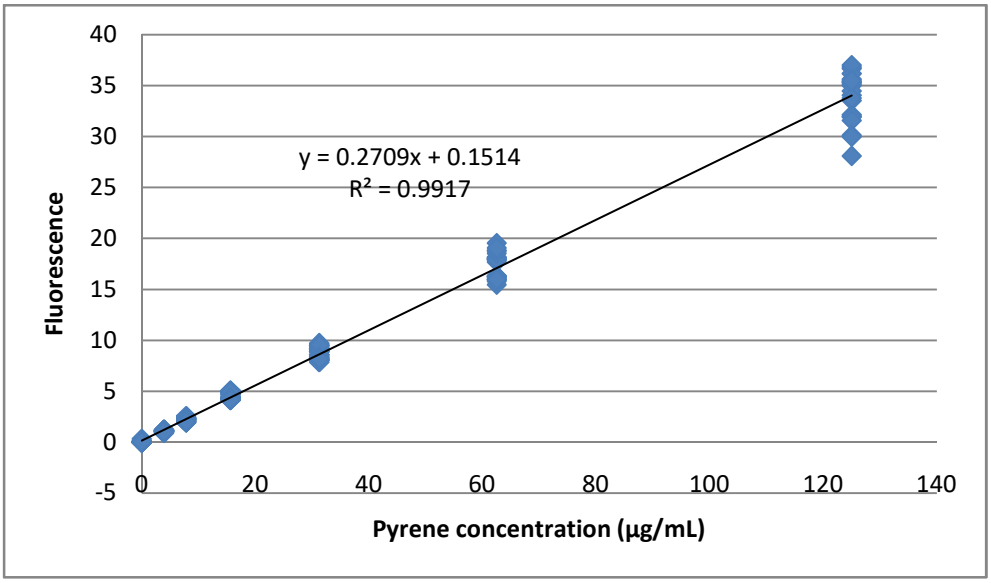

Figure 2-3 - Fluorescence intensity using a range of concentrations of 3.91 until 125 µg/mL pyrene. Each concentration of pyrene was determined 4 times and for each determination 4 replicates were done, including a control, with no contaminant added. Linear adjustment (R^2) and equation are shown in the figure. Linear equation was used for the calculation of naphthalene equivalents in samples.

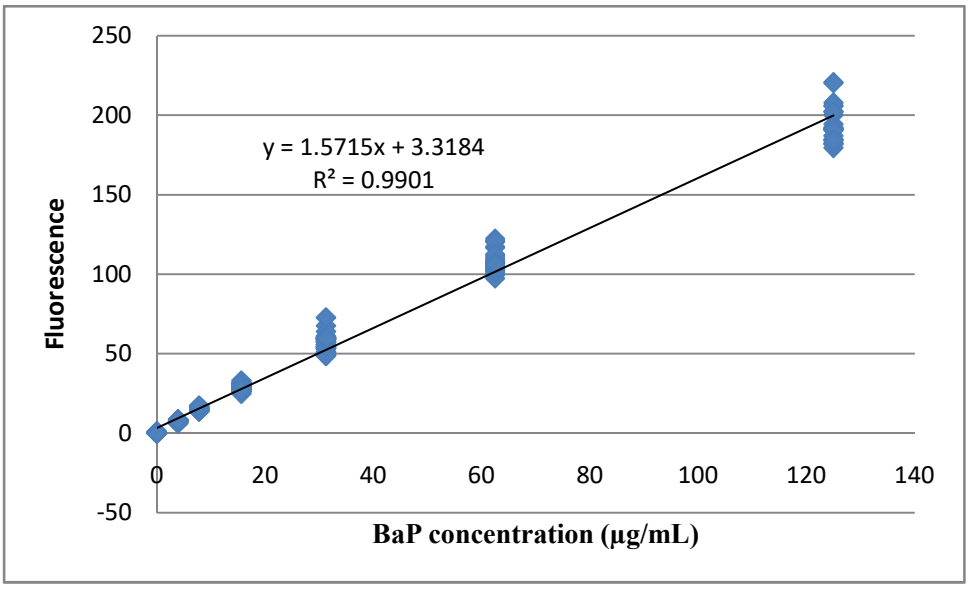

Figure 2-4 - Fluorescence intensity using a range of concentrations of 3.91 until 125 µg/mL benzo[a]pyrene (BaP). Each concentration of BaP was determined 4 times and for each determination 4 replicates were done, including a control, with no contaminant added. Linear adjustment (R^2) and equation are shown in the figure. Linear equation was used for the calculation of naphthalene equivalents in samples.

2.2.4 Limit of Detection (LOD) and Limits of Quantification (LOQ)

Limits of detection (LOD) and limits of quantification (LOQ) were calculated for the present methodology for the different PAHs equivalents measured as described by (Shrivastava and Gupta, 2011). For naphthalene equivalents the LOD obtained was 23.55 µg/mL and the LOQ set was 78.51 µg/mL. For phenanthrene equivalents LOD and LOQ calculated were 0.602 µg/mL and 2.01 µg/mL, respectively. For pyrene equivalents LOD and LOQ calculated were 0.174 µg/mL and 0.579 µg/mL. Lastly, for Benzo[**a**]pyrene equivalents a LOD of 0.004 µg/mL and a LOQ of 0.014 µg/mL were obtained.

2.2.5 Cotinine levels in parturient

Cotinine, a metabolite of nicotine, was measured in the plasma of parturient in order to assess tobacco smoke exposure. The quantification was performed using a commercial ELISA Kit (Sigma Aldrich) following the instructions of the manufacturer.

2.2.6 Statistics analysis

Non-parametric tests were performed since data did not present normality and/or homoscedasticy (Shapiro-Wilk tests). Spearman correlation analysis was carried out between PAHs equivalents levels in the different matrices analyzed, namely in the placenta (homogenized and supernatant), blood cells (from mother and umbilical cord) and plasma (mother and umbilical cord). A Spearman correlation analysis was also performed between cotinine level in the parturient plasma and levels of PAHs equivalents in all biological matrices. To test for significant differences between groups (related to smoking habits, area of residence, newborns weight, cephalic perimeter and length) a student t-test (for 2 groups) or a One-Way ANOVA (3 groups) analysis was performed. Post-hoc tests were performed when necessary, i.e. Dunn's test. Statistical analyses were performed using the software SigmaPlot for Windows, version 12.0.

Potential influence variables (e.g. lifestyle, smoking habits, area of residence and others) on the retrieved PAHs levels in placenta and blood matrices were analyzed using redundancy analysis (RDA). Forward selection was used in RDA for the selection of the subset of variables giving the best explanatory model. A Monte Carlo permutation test using 499 permutations was used to calculate the significance of each explanatory variables. The environmental factors with $p<0.05$ were included in the constrained ordination model. The analysis was carried out by CANOCO 4.5 software.

2.3. Results

2.3.1 Demographic Characteristics

Socio-demographic and clinical characteristics from 49 mother/newborn pairs enrolled in this study were resident in 11 municipalities from Aveiro region: 18 from Aveiro city, 9 from Ílhavo, 5 from Oliveira do Bairro, 4 from Estarreja, 4 from Murtosa, 3 from Águeda, 3 from Abergaria-a-Velha, 2 from Vagos and 1 from Ovar (Table 2-1). From all participants, according the questionnaire, 41% of the participants lived in urban areas and 35% in rural areas (Table 2-S1). The remaining participants lived in areas classified as intermediate, between urban and rural areas according the questionnaire answers (Table 2-S1). Other socio-demographic data can be found in Table 2-1.

Table 2-1 - Socio-demographic and clinical characteristics of mother newborn pairs.

Clinical and Demographic Characteristics	MEAN ± SD OR %	N
Age (Years)	30.1±5.8	49
BMI before pregnancy (kg/m^2)	29.6±4.2	49
Municipality of residence (%)		
Aveiro	36.7	18
Ílhavo	18.4	9
Oliveira do Bairro	10.2	5
Estarreja	8.2	4
Murtosa	8.2	4
Águeda	6.1	3
Albergaria-a-Velha	6.1	3
Vagos	4.1	2
Ovar	2.0	1
Time of residence (years)	17.3±12.4	49
Education Level (%)		
Primary incomplete	4.1	2
Primary complete	10.2	5
High School	49.0	24
University	36.7	18

2.3.2 PAHs in placenta and blood

Values of PAHs equivalents levels below LOD were not included in data analysis. In general, the four PAHs levels were significantly higher in the fraction of homogenized placenta than in the umbilical cord blood and mothers' blood fractions (plasma or blood cells) analyzed (Dunn's post-hoc test; $p<0.05$; Tables 2-2, 2-3, 2-4 and 2-5.). Considering both fractions assessed in placenta, no significant differences on PAHs levels were obtained between the supernatant fraction and the homogenate fraction ($p>0.05$). Furthermore, each PAH-type equivalents determined in the umbilical cord blood fractions (plasma or blood cells) presented similar levels when compared to mother blood fractions (Dunn's post-hoc test; $p>0.05$).

Table 2-2 - Naphthalene equivalents levels above LOD determined in biological matrices: placenta, umbilical cord blood and parturient blood. Values reported correspond to the average, standard deviation (SD), median, percentiles (P25 and P75), minimum (Min), maximum (Max) and N are presented. Values are expressed in µg/mg protein.

Naphthalene	N	Average	SD	Median	P25	P75	Max	Min
Homogenized placenta	49	8.03[a]	3.24	7.25	6.09	9.62	18.49	2.21
Umbilical cord blood cells	14	0.10[b]	0.03	0.09	0.08	0.11	0.15	0.05
Parturient blood cells	4	0.09[b]	0.01	0.09	0.11	0.08	0.08	0.10

[a,b]. Different letters represent significant differences (Dunn's post-hoc; $p<0.05$).

Table 2-3 - Phenanthrene equivalents levels above LOD determined in biological matrices: placenta, umbilical cord blood and parturient blood. Values reported correspond to the average, standard deviation (SD), median, percentiles (P25 and P75), minimum (Min), maximum (Max) and N are presented. Values are expressed in ng/mg protein.

Phenanthrene	N	Average	SD	Median	P25	P75	Max	Min
Homogenized placenta	49	285[a]	125	264	201	353	690	48
Supernatant Placenta	6	44[a,b]	19.44	37.85	32.6	45.95	82.1	30.5
Umbilical cord blood cells	9	2.98[c]	0.735	2.65	2.34	3.57	4.13	2.23
Umbilical cord plasma	32	8.93[c,d]	3.84	7.72	6.61	9.7	2.43	5.34
Parturient plasma	36	12.77[b,d]	4.73	11.35	9.80	14.83	29.8	7.51

[a,b,c,d] Different letters represent significant differences (Dunn's post-hoc test; $p<0.05$).

Table 2-4 - Pyrene equivalents levels above LOD determined in biological matrices: placenta, umbilical cord blood and parturient blood. Values reported correspond to the average, standard deviation (SD), median, percentiles (P25 and P75), minimum (Min), maximum (Max) and N are presented. Values are expressed in ng/mg protein.

Pyrene	N	Average	SD	Median	P25	P75	Max	Min
Homogenized placenta	10	23.22[a]	16.81	16.8	12.98	26.88	65.9	10.8
Supernatant placenta	8	15.41[a]	6.14	13.9	10.9	17.33	28.2	9.55
Umbilical cord plasma	9	3.66[b]	2.83	2.64	2.24	3.30	10.2	0.85
Parturient plasma	13	4.06[b]	2.92	2.92	2.36	3.86	12.5	2.02

[a,b] Different letters represent significant differences (Dunn's post-hoc test; $p<0.05$).

Table 2-5 - Benzo[a]pyrene (BaP) equivalents levels above LOD determined in biological matrices: placenta, umbilical cord blood and parturient blood. Values reported correspond to the average, standard deviation (SD), median, percentiles (P25 and P75), minimum (Min), maximum (Max) and N are presented. Values are expressed in ng/mg protein.

BaP	N	Average	SD	Median	P25	P75	Max	Min
Homogenized placenta	13	3.18[a]	2.82	2.06	1.25	3.29	8.39	0.53
Supernatant placenta	5	2.73[a]	2.34	2.67	2.83	2.83	6.53	0.73
Umbilical cord plasma	13	0.39[b]	0.24	0.29	0.21	0.58	0.81	0.08
Parturient plasma	14	0.40[b]	0.36	0.29	0.15	0.54	1.28	0.06

[a,b] Different letters represent significant differences (Dunn's post-hoc test; $p<0.05$).

Naphthalene equivalents measured in the homogenized placenta were not significantly correlated with those measured in the umbilical cord cells ($r=0.216$; $p=0.136$) (data not shown). No other correlations were performed for naphthalene equivalents in other matrices since they were below LOD (data not shown). Considering levels of phenanthrene equivalents measured in the different matrices studied, the only significant correlation found was between their levels in umbilical cord plasma and mother plasma (Table 2-6.).

Levels of pyrene equivalents and benzo[**a**]pyrene equivalents quantified in the different placenta (homogenate and supernatant) and plasma (mother and umbilical cord) fractions were all significantly correlated with each other ($p<0.05$; Table 2-7 and 2-8), with the exception between levels of benzo[**a**]pyrene equivalents in homogenized placenta and their respective supernatant ($p>0.05$).

Table 2-6 - Spearman correlation analysis between the levels of phenanthrene equivalents in placenta (homogenate and supernatant fractions), umbilical cord blood (cells and plasma) and mothers' plasma. Values refer to Spearman correlation coefficient (r) and respective p values (between brackets).

Phenanthrene	Homogenized placenta	Supernatant placenta	Umbilical cord blood cells	Umbilical cord plasma	Parturient plasma
Homogenized placenta		0.0269 (0.854)	0.259 (0.0754)	0.0771 (0.597)	0.177 (0.223)
Supernatant placenta			-0.0307 (0.835)	0.241 (0.0951)	0.211 (0.146)
Umbilical cord blood cells				0.00528 (0.971)	-0.0816 (0.580)
Umbilical cord plasma					**0.578 (<0.001)**
Parturient plasma					

Table 2-7 - Spearman correlation analysis between the levels of pyrene equivalents in placenta (total homogenate and supernatant fractions), umbilical cord blood plasma and mothers' plasma. Values refer to Spearman correlation coefficient (r) and respective p values (between brackets).

Pyrene	Homogenized placenta	Supernatant placenta	Umbilical cord plasma	Parturient plasma
Homogenized placenta		**0.630 (<0.001)**	**0.582 (<0.001)**	**0.374 (<0.001)**
Supernatant placenta			**0.713 (<0.001)**	**0.507 (<0.001)**
Umbilical cord plasma				**0.604 (<0.001)**
Parturient plasma				

Table 2-8 - Spearman correlation analysis between the levels of benzo[a]pyrene (BaP) equivalents in placenta (homogenate and supernatant fractions), umbilical cord blood plasma and mothers' plasma. Values refer to Spearman correlation coefficient (r) and respective p values (between brackets).

BaP	Homogenized placenta	Supernatant placenta	Umbilical cord plasma	Parturient plasma
Homogenized placenta		0.243 (0.0924)	**0.673 (<0.001)**	**0.472 (<0.001)**
Supernatant Placenta			**0.509 (<0.001)**	**0.503 (<0.001)**
Umbilical cord plasma				**0.756 (<0.001)**
Parturient plasma				

2.3.3 Anthropometric data of newborns and PAHs levels in placenta and blood

Body weight and head circumference at birth was significantly smaller in female newborns than in male newborns (t-student test; $p<0.05$) (Table 2-9.). Newborn males at birth had an average length of 50 cm (±1.4), weight 3319.4 g (± 381.4) and head circumference of 34.9 (±0.92), whereas females had a birth length of 49 cm (±1.6) and weight of 3135.1 g (±369.2) and head circumference of 33.9 (± 0.83) . There were no significant correlations between the levels of naphthalene, phenanthrene, pyrene and BaP equivalents measured in placenta and anthropometric data of newborns (data not shown). Despite that, is noteworthy that almost all correlation coefficients obtained were negative.

Pyrene equivalent content in homogenized placenta was significantly higher ($p=0.042$) in the group of newborns that presented a weight at birth below 3 kg. Moreover, levels of BaP equivalent in placenta were higher ($p=0.05$) in newborns with a length below 35 cm at birth. Although no significant correlations were established, it was possible to observe patterns for higher levels of naphthalene and phenanthrene equivalents when newborns had lower weight, length and cephalic perimeter at birth (Table 2-10).

Table 2-9 - Anthropometric data of the newborns.

Birth outcomes	AVERAGE ± SD or %	N
Gender (%)		
Male	51.0	25
Female	49.0	24
Birth length (cm)		
Male	49.9±1.4	25
Female	49.0±1.6	24
Birth weight (g)		
Male	3319.4±381.4	25
Female	3135.1±369.2	24
Head circumference (cm)		
Male	34.9±0.92	25
Female	33.9±0.83	24

Table 2-10 - Naphthalene, phenanthrene, pyrene and benzo[a]pyrene equivalents levels in placenta (homogenate fraction) grouped by newborns weight, cephalic perimeter and length. SD-Standard deviation. Values represent average ± standard deviation and are expressed in µg/mg protein.

	Naphthalene	Phenanthrene	Pyrene	Benzo[a]Pyrene
Newborn weight (Kg)				
>3kg	7.74±3.03	0.27±0.13	0.01±0.003	$3.27x10^{-03}$ ±0.003
≤3kg	8.27±3.72	0.30±0.13	0.04±0.02 *	$3.05x10^{-03}$ ±0.002
Cephalic perimeter (cm)				
≥35	7.47±3.51	0.26±0.14	0.01±0.01	0.004±0.004
<35	8.09±3.28	0.28±0.12	0.02±0.02	0.003±0.003
Newborn Length (cm)				
≥50	7.38±2.09	0.25±0.10	0.01±0.01	0.002±0.003
<50	8.35±4.21	0.29±0.15	0.02±0.02	0.004±0.003*

* represent significant differences between the groups (t-student test; $p<0.05$).

2.3.4 Smoking habits and PAHs in placenta and blood

The levels of naphthalene, phenanthrene and benzo[**a**]pyrene equivalents in placenta (homogenate fraction) were higher in parturient that reported who exhibit smoking habits during and/or before pregnancy (Table 2-11), although no significant differences were registered (t-student test; $p>0.05$). Only six of the forty nine parturient smoked during the last trimester of pregnancy (1-10 cigarettes per day). Significant correlations were obtained between phenanthrene equivalents determined in parturient's plasma and cotinine levels (Table 2-S2; $r=-0.464$; $p=0.005$) and the correlation of cotinine levels with levels of phenanthrene equivalents in the supernatant fraction of placenta was attained ($p=0.05$; $r=0.829$). No significant correlations were obtained between the naphthalene, pyrene and BaP equivalents levels in the biological matrices and cotinine levels measured in plasma of parturient.

Table 2-11 - Naphthalene, phenanthrene, pyrene and benzo[a]pyrene equivalents levels in placenta of smoker and non-smoker parturient (before and/or during pregnancy). Values are presented as average ± standard deviation and are expressed in µg/mg protein.

Homogenized Placenta	Naphthalene	Phenanthrene	Pyrene	BaP	N (%)	N
Smoke						
Yes	8.23±4.35	0.86±2.39	0.01±0.002	$3.96x10^{-03}$ ±0.004	34.5 %	17
No	7.92±2.55	0.28±0.10	0.03±0.02	$3.04x10^{-03}$ ±0.003	65.3 %	32

However, levels of naphthalene equivalents in homogenized placenta revealed significant differences between the different groups of parturient classified according to their type of passive smoking in the work place (never, sporadically, daily (1hour); daily (more than 3 hours); one-way ANOVA; $p<0.05$). Similarly, levels of phenanthrene equivalents were also significantly different in groups referring different passive smoking habits at home (one-way ANOVA; $p>0.05$; Table 2-S3). However, in both cases, Dunn's post-hoc testing did not revealed statistical differences between the groups. Furthermore, according to the RDA analysis performed (Figure 2-5), the levels of benzo[**a**]pyrene equivalents in placenta and plasma (mother and umbilical cord) were positively correlated with the exposure to smoke at work ($F=3.055$; $p=0.0460$; Figure 2-5).

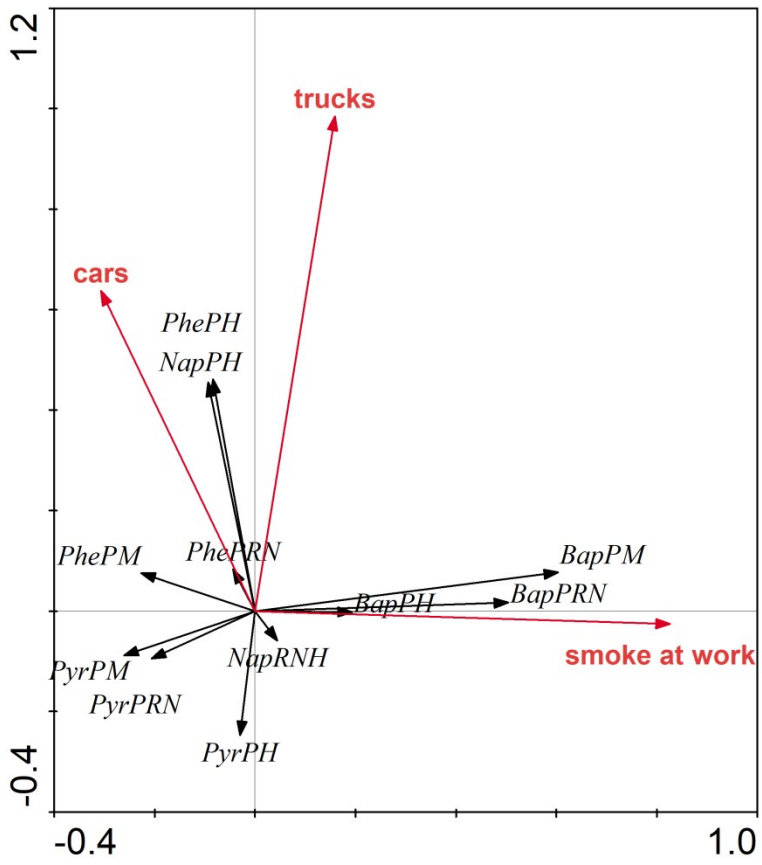

Figure 2-5 - Biplots based on redundancy analysis (RDA) representing the correlation between naphthalene, phenanthrene, pyrene and BaP equivalents levels in biological matrices (blood and placenta) and significant influence variables (parturient passive smoking at work and residence near roads, highlighting cars and/or heavy vehicles traffic like trucks).

The maximum levels of naphthalene (18.49 µg/mg protein) and phenanthrene equivalents (0.69 µg/mg protein) registered were found in placenta of parturient who smoked in the last trimester of pregnancy. The levels of naphthalene, phenanthrene and BaP equivalents were in fact higher in homogenized placenta of women that smoked in last trimester of pregnancy, but no significant differences were registered ($p>0.05$) (Figure 2- 6.).

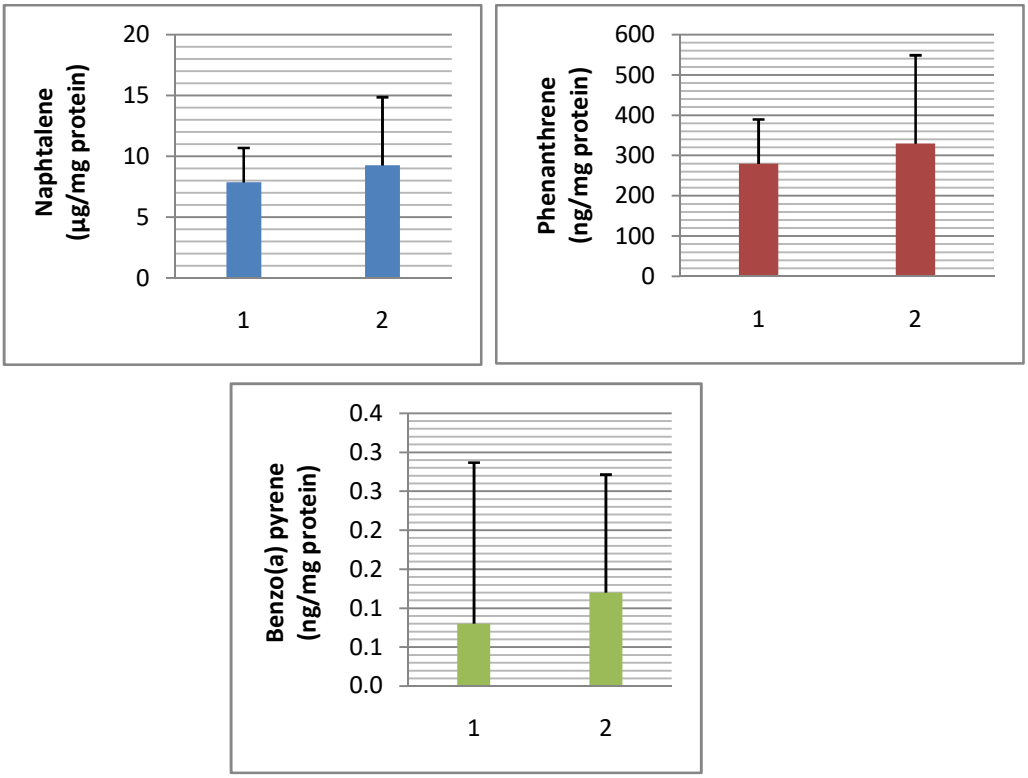

Figure 2-6 - Naphthalene, phenanthrene and benzo[a]pyrene equivalents levels in placenta of non-smoker (1) and smoker (2) parturient during the 3rd trimester of pregnancy. Values represent mean ± standard deviation and are expressed in µg/mg protein.

2.3.5 Area of parturient residence

Low molecular weight PAHs (naphthalene and phenanthrene equivalents) and pyrene equivalents measured in homogenized placenta presented relatively higher levels (non-significant differences; $p>0.05$; Table 2-S1) in parturient living in rural locations. Parturient living nearby traffic roads, where cars and/or heavy road vehicles were constantly passing, also presented relatively high levels of PAHs equivalents (Table 2-S1). Namely, LPAHs in homogenized placenta presented significant differences between the different groups of parturient taking into account the presence of heavy road vehicles nearby their residence (one-way ANOVA for naphthalene equivalents, $p=0.03$; one-way ANOVA for phenanthrene equivalents; $p=0.041$) (Table 2-S1), although Dunn's post-hoc testing did not revealed statistical differences between those groups. According to the RDA analysis (Figure 2-5) the levels of phenanthrene equivalents in homogenized placenta and plasma (mother and umbilical cord) and of naphthalene equivalents in homogenized placenta were positively related with vehicle

exhausts, namely with areas of parturient residence next to roads where cars and/or heavy vehicles pass constantly (trucks; RDA analysis, F=2.446; p=0.0280 and cars; RDA analysis, F=2.302; p=0.0320). Further details concerning parturient work place and chemicals exposure at work are presented in Table 2-S4.

2.3.6 PAHs levels in placenta along Aveiro district

In Tables 2-12, 2-13, 2-14 and 2-15 levels of the 4 PAHs types in placenta are displayed according to the county of parturient's residence in Aveiro district. No significant differences were registered between the levels of PAHs from parturient of the different counties ($p>0.05$). However, it is noteworthy that naphthalene and phenanthrene equivalents presented the highest levels in parturient from Aveiro and Ílhavo counties (Tables 2-12 and 2-13). Due to the fact that pyrene and benzo[**a**]pyrene equivalents in homogenized placenta were below the detection limits in samples from some counties, the respective means are not presented, or presented with a reduced number of samples (Tables 2-14 and 2-15).

Table 2-12 - Levels of naphthalene equivalents in homogenized placenta of 49 mother/newborn pairs from different municipalities of the Aveiro district, Portugal. Values represent mean ± standard deviation and are expressed in µg/mg protein.

County of residence	Number of parturient (N)	Naphthalene equivalents (Mean ± SD)
Aveiro	18	8.61±3.61
Ílhavo	9	8.60±4.13
Oliveira do Bairro	5	7.65±2.43
Estarreja	4	8.26±1.95
Murtosa	4	6.77±1.46
Águeda	3	5.27±2.67
Albergaria-a-Velha	3	8.30±5.00
Vagos	2	7.65
Ovar	1	6.80

Table 2-13 - Levels of phenanthrene equivalents in homogenized placenta of 49 mother/newborn pairs from different municipalities of the Aveiro district, Portugal. Values represent mean ± standard deviation and are expressed in µg/mg protein.

Demographic Characteristics (Residence)	Number of parturient (N)	Phenanthrene equivalents (Mean ± SD)
Aveiro	18	0.30±0.15
Ílhavo	9	0.31±0.16
Oliveira do Bairro	5	0.27±0.09
Estarreja	4	0.28±0.07
Murtosa	4	0.24±0.07
Águeda	3	0.20±0.07
Albergaria-a-Velha	3	0.30±0.20
Vagos	2	0.28
Ovar	1	0.23

Table 2-14 - Levels of pyrene equivalents in homogenized placenta of 49 mother/newborn pairs resident in different municipalities of the Aveiro district, Portugal. Values represent mean ± standard deviation and are expressed in µg/mg protein.

Demographic Characteristics (Residence)	Number of parturient (N)	Pyrene equivalents levels (Mean ± SD)
Aveiro	5	0.02±0.009
Ílhavo	1	0.03
Oliveira do Bairro	1	0.01
Águeda	2	0.04
Ovar	1	0.01

Table 2-15 - Levels of benzo[a]pyrene (BaP) equivalents in homogenized placenta of 49 mother/newborn pairs inform different municipalities of the Aveiro district, Portugal. Values represent mean ± standard deviation and are expressed in µg/mg protein.

Demographic Characteristics (Residence)	Number of parturient (N)	BaP equivalents (Mean ± SD)
Aveiro	2	0.005
Ílhavo	5	0.002±0.001
Estarreja	2	0.005
Murtosa	2	0.002
Águeda	1	0.007
Vagos	1	0.0005

2.4. Discussion

2.4.1 PAHs in Placenta and blood

In the present study, levels of PAHs equivalents in placenta were in general higher than in blood cells (mother and umbilical cord) or plasma (mother and umbilical cord). In fact, placenta can act as a barrier to xenobiotics, including PAHs, partially preventing them to reach the fetus. Due to their lipophilic nature, PAHs can penetrate though the cells membranes, including those of placenta leading to PAHs accumulation (Myllynen, Pasanen and Pelkonen, 2005; Kumar *et al*., 2014). In this study, phenanthrene, pyrene and BaP equivalents presented similar content in mothers' plasma and in the umbilical cord plasma, suggesting that placenta indeed act as a partial but not complete barrier for this type of PAHs. Several studies have reported PAHs levels in these maternal-fetus matrices, depending on the exposure scenarios. Some studies show evidence of patterns similar to the ones of the present study, with BaP equivalents showing lower concentrations in umbilical cord blood when compared to the placenta measurements (e.g. Madhavan and Naidu, 1995; Yu *et al*., 2011). Another finding that can relate to the barrier role of placenta is the detection of BaP equivalents at higher levels in mothers' plasma than umbilical cord plasma (Radmacher, Looney and Myers, 2010), showing that partly is retained in placenta.

Phenanthrene, pyrene and BaP equivalents levels in blood cells were bellow LOD. It has been suggested that plasma lipidic properties make PAHs to bind preferably to the plasma monolayer (Massey and Pownall, 1998). A similar pattern has been described in a study where the major part of POPs were found in plasma (more than 50%) when compared with blood cells (Kärrman *et al*., 2006).

Patterns for PAHs accumulation may also depend on the molecular structure, i.e. the number of rings present. In the present study, LPAHs levels were in general higher than HPAHs contents in tissues, which is in accordance with the study of Zhang *et al*., (2017), where LPAHs levels were higher in placenta, umbilical cord blood and mothers' blood when compared to HPAHs.

This pattern may be related to the emission and exposure type of different PAHs, where LPAHs are generally found in gaseous phases of exposure, while HPAHs have a

bigger size and are found adsorbed to the particulate phase of exposure (e.g. through fly ash and soot). Therefore, their entrance in the body and consequently their bioaccumulation will differ (Abdel-Shafy and Mansour, 2015). Moreover, some LPAHs species have a similar structure to steroid hormones like E2 (Yin *et al*., 2016), which can enable and facilitate their entrance in organs like the placenta (Yin *et al*., 2016).

Even though presenting higher level, no correlation was attained between levels of LPAHs in the placenta and blood. So, LPAHs levels in the matrices represent a different organ and tissue. On the other hand, HPAHs levels in placenta were correlated with blood from both mother and the umbilical cord. Therefore, if this pattern is confirmed for other similar studies, PAHs measurements in mothers' blood may be replaced by placenta with the advantage of being considered a non-invasive matrix.

Several other studies have used the placenta as a matrix for PAHs measurements (e.g. Gladen *et al*., 2000; Yuan *et al*., 2013), although the use of different methodologies and analytical techniques make comparisons difficult. In addition, the use of different biological matrices (e.g. different tissues, organs or excretions) dependent on the aims of the studies make somehow difficult comparing exposure scenarios for different population (Sexton *et al*., 2011). Furthermore, there are no recommended threshold levels for PAHs in the human matrices analyzed in the present study. However, there is an indicative level for PAHs in urine to characterize parturient exposure to PAHs and to complement data obtained in placenta and blood matrices. A metabolite of pyrene quantified in urine (1OH-pyrene) is one of the most used biomarkers for PAHs exposure. American Conference of Governmental Industrial Hygienists (ACGIH, 2010) uses this non-invasive matrix to assess total burden of PAHs levels in occupational groups with an indicative of 0.5 µmol 1OH-Pyrene /mol creatinine in urine.

2.4.2 Exposure to tobacco smoke

Lifestyle during pregnancy can reflect on fetus exposure to xenobiotics. If the mother is a smoker, the fetus can be exposed to different contaminants, including PAHs (Sexton *et al*., 2011). In this study, naphthalene, phenanthrene and BaP equivalents levels in homogenized placenta from pregnant women who smoked during the 3rd trimester were higher than from those who did not smoke during this period. The same occurred for parturient who have smoked throughout their live time. These mothers'

presented higher naphthalene, phenanthrene and BaP equivalents contents comparing to the non-smoking women. Furthermore, according to the redundancy analysis there was a significant association between BaP equivalents levels in placenta and plasma (both from mother and umbilical cord) and passive smoke at work. Similar findings were observed in the study from Machado *et al*. (2014), with parturient from Porto Alegre, Brazil, where higher BaP levels in umbilical cord blood were related to smoking habits.

Cotinine, a metabolite of nicotine, is a commonly used marker for smoking habits (Barr, Bishop and Needham, 2007). Contrarily to what was expected, in the present study no significant correlations were found between cotinine measured in parturient plasma and PAHs levels in placenta and blood matrices. Similarly Yuan *et al*. (2013) reported no association between PAH-DNA adducts in placenta and nicotine and cotinine in blood.

2.4.3 Influence of traffic exhausts

It is known that, PAHs like naphthalene- and phenanthrene-type PAHs can occur through anthropogenic emissions like coal tar, gasoline or diesel fuel combustion (Lai *et al*., 2011), generally presented in the gaseous phase of emissions and exposures (Kim *et al*., 2013). In the present study, higher content of LPAHs and pyrene equivalents were found in placenta from parturient resident in rural areas. However, naphthalene and phenanthrene equivalents levels were higher in parturient inhabiting next to roads with high traffic (cars), also confirmed by the redundancy analysis. Although rural exposure was not expected, we cannot disregard that women may work in city centers and be exposed during the day to this type of emissions. According to Zhang, Zhang and Wang (2009) that discriminated the sources of 16 PAHs, it is possible to relate LPAHs like naphthalene and phenanthrene equivalents with coal tar combustion, specifically. According to Albuquerque, Coutinho and Borrego (2016) motor vehicles may cause an increase of PAHs in the environment.

2.4.4 Influences of PAHs in anthropometric data of newborns

High PAHs levels in the body can be one of the consequences for length, weight and head circumference diminution, preterm and intrauterine growth restrictions and other major problems like neural tube defects or abortion *(*Ren *et al*., 2011; Chen *et al*., 2014). In the studied population at Aveiro, parturient with newborns with less weight

had significant higher pyrene equivalents levels in homogenized placenta, while lower length newborns had significant higher BaP equivalents levels in homogenized placenta. Higher (but non-significant) levels of naphthalene, phenanthrene, pyrene and BaP equivalents in placenta were also found in newborns with smaller head circumference. According to Choi *et al*. (2006), PAHs higher concentration found in umbilical cord blood was associated with significantly reduced weight in the Krakow Caucasians and New Yorkers African Americans too. Additionally, Jedrychowski *et al*. (2013) revealed that prenatal airborne and dietary PAHs concentration exposure may be a possibly cause to the lower birth weight of the newborns in Poland. In Beijing (China) a study with 130 patients (80 patients with neural tube defects and 50 control patients) that revealed that the higher PAHs means concentrations were found in patients placenta tissue with a serious birth defect than the PAHs concentration mean in placenta tissue of patients without the problem (Ren *et al*., 2011). Al-Saleh *et al*. (2013) present that benzo[**b**]fluoranthene, benzo[**a**]pyrene, chrysene, benzo[**a**]anthracene and dibenzo[**a,h**]anthracene equivalents levels in blood (mothers' and umbilical cord) and placenta may affect the anthropometric data (weight, length and head circumference). It is difficult to compare measured PAHs levels in umbilical cord blood, mothers' blood and placenta because of the different analytical techniques and unities used in different studies (Sexton *et al*., 2011).

2.4.5 PAHs levels along Aveiro region

Considering naphthalene and phenanthrene levels in placenta of parturient resident in different counties of the Aveiro region, no significant differences were found between LPAHs levels. The high variability of data and low numbers of parturient from some of the counties contribute to these non-significant results. Noteworthy, a trend was found in LPAHs levels. Naphthalene and phenanthrene equivalents were higher in Aveiro, Ílhavo and Albergaria-a- Velha and lower in Águeda counties. This could occur because Aveiro and Ílhavo are counties with larger urban areas. However, the counties of Abergaria-a-Velha and Águeda have larger rural areas and they are both very industrialized. Despite there are several studies that reveal that PAHs concentrations are higher in urban areas atmosphere than in rural places (e.g. Ravindra, Sokhi and Van Grieken, 2008; Zhang and Tao, 2009; Wang, Zhu and Chen, 2013).

In this study, the majority of placenta samples presented levels of HPAHs (pyrene and benzo[**a**]pyrene equivalents) below LOD and due to the low number of replicates above LOD in each county, no comparisons were performed between Aveiro counties.

2.5. Conclusion

In conclusion, placenta plays a role as a barrier for PAHs content although it is not fully effective, as PAHs levels are still found in blood from the umbilical cord. In Aveiro region, the highest PAHs concentration was found in homogenized placenta comparatively to the other matrices. The lowest PAHs concentration was found in umbilical cord blood. In this study, the parturient living in Aveiro, Ílhavo and Albergaria-a-Velha had the highest concentration of PAHs in the homogenized placenta, with Águeda presenting the lowest PAHs concentrations in the same matrix. Other demographic and environmental data which may be related to high PAHs concentrations in homogenized placenta is the proximity of residence to roads with high traffic. In concern to smoke habits, naphthalene, phenanthrene and BaP equivalents levels present in homogenized placenta were higher in parturient with smoking habits during the last trimester of pregnancy. Although non-significant, alterations in anthropometric data were observed in newborns and may be related with higher levels of PAHs, with decreases on weight, length and head circumference. Parturient who had newborns with less weight had significant higher pyrene equivalents levels in homogenized placenta, while smaller newborns presented significant higher BaP levels homogenized placenta.

2.6. References

Abdel-Shafy, H.I. and Ma N.nsour, M. 2015. "A Review on Polycyclic Aromatic Hydrocarbons: Source, Environmental Impact, Effect on Human Health and Remediation." *Egyptian Journal of Petroleum* 25 (1). Egyptian Petroleum Research Institute: 107–23. doi:10.1016/j.ejpe.2015.03.011.

ACGIH, 2010. Documentation for a Recommended BEI of Polycyclic Aromatic Hydrocarbons. American Conference of Governmental Industrial Hygienists, Cincinatti, Ohio, USA.

Al-Saleh, I., Alsabbahen,A., Shinwari, N., Billedo, G., Mashhour, A., Al-Sarraj, Y., El Din Mohamed, G. and Rabbah, A. 2013. "Polycyclic Aromatic Hydrocarbons (PAHs) as Determinants of Various Anthropometric Measures of Birth Outcome." *Science of the Total Environment* 444. Elsevier B.V.: 565–78. doi:10.1016/j.scitotenv.2012.12.021.

Albuquerque, M., Coutinho, M. and Borrego, C. 2016. "Science of the Total Environment Long-Term Monitoring and Seasonal Analysis of Polycyclic Aromatic Hydrocarbons (PAHs) Measured over a

Decade in the Ambient Air of Porto , Portugal." *Science of the Total Environment, The* 543. Elsevier B.V.: 439–48. doi:10.1016/j.scitotenv.2015.11.064.

Alves, A.C., Monteiro M.S., Machado, A.L., Oliveira, M., Bóia, A., Correia, A., Oliveira,N., Soares, A.M.V.M., Loureiro, S. 2017. "Mercury Levels in Parturient and Newborns from Aveiro Region , Portugal Mercury Levels in Parturient and Newborns from Aveiro Region , Portugal." *Journal of Toxicology and Environmental Health, Part A* 0 (0). Taylor & Francis: 1–13. doi:10.1080/15287394.2017.1286926.

Annamalai, J., and Namasivayam, V. 2015. "Endocrine Disrupting Chemicals in the Atmosphere : Their Effects on Humans and Wildlife." *Environment International* 76. Elsevier Ltd: 78–97. doi:10.1016/j.envint.2014.12.006.

Baglan R.J., Brill A.B., Schulert D., Wilson D., Larsen D., Dryer N., Mansour, M., Schaffner, W., Hoffman, L., Davies, J. 1974. Utility of placenta tissue as an indicator of trace element exposure to adult and fetus. Environ Res;8:64–70.

Barr, D. B., Bishop, A.and Needham L. 2007. "Concentrations of Xenobiotic Chemicals in the Maternal-Fetal Unit." *Reproductive Toxicology* 23: 260–66. doi:10.1016/j.reprotox.2007.03.003.

Bauer, M. K., Harding, J. E., Bassett, N. S., Breier, B. H., Oliver, M. H., Gallaher, B. H., Evans, P. C., Woodall, S. M. and Gluckman, P. D. 1998. "Fetal Growth and Placental Function." *Molecular and Cellular Endocrinology* 140: 115–20.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*(1-2), 248-254.

Chen, Q., Zheng, T., Bassig, B., Cheng, Y., Leaderer, B., Lin, S., Holford, T., Qiu, J., Zhang, Y., Shi,K., Zhu, Y., Niu,J.,Li, Y., Guo, H., Hu, X., Jin, Y. 2014. "Prenatal Exposure to Polycyclic Aromatic Hydrocarbons and Birth Weight in China." *Open Journal of Air Pollution* 3 (December): 100–110. doi:10.4236/ojap.2014.34010.

Choi, H., Wang, L., Lin, X., Spengler, J. and Perera, F. P. 2012. "Fetal Window of Vulnerability to Airborne Polycyclic Aromatic Hydrocarbons on Proportional Intrauterine Growth Restriction." *PLoS ONE* 7 (4). doi:10.1371/journal.pone.0035464.

Gladen, B. C., Zadorozhnaja, T. D., Chislovska, N., Hryhorczuk, D. O., Kennicutt M. C. and Little R. E. 2000. "Polycyclic Aromatic Hydrocarbons in Placenta," 597–603.

Gravato, C. and Santos, M. A. 2003. "Genotoxicity Biomarkers' Association with B(a)P Biotransformation in Dicentrarchus Labrax L." *Ecotoxicology and Environmental Safety* 55 (3): 352–58. doi:10.1016/S0147-6513(02)00070-2.

International Agency of Research on Cancer (IARC), 2010. Some Non heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 92. World Health Organization, Lyon, France, pp. 1e853.

Jedrychowski, W., Perera, F., Tang , D., Rauh, V., Majewska R., Mroz E., Flak E. Stigter, L., Spengler, J. , Camann, D. and Jacek, R. 2013. "The relationship between prenatal exposure to airborne polycyclic aromatic hydrocarbons (pah) and pah-dna adducts in cord blood." *Journal of Exposure Science and Environmental Epidemiology* 23 (4): 371–77. doi:10.1038/jes.2012.117.THE.

Jeong, Y., Lee, S., Kim, S., Park, J., Kim, H.-J., Choi, G., Choi S., Kim S., Kim S.Y., Kim, S., Choi, K., Moon, H.-B. 2018. "Science of the Total Environment Placental Transfer of Persistent Organic Pollutants and Feasibility Using the Placenta as a Non-Invasive Biomonitoring Matrix." *Science of the Total Environment* 612. Elsevier B.V.: 1498–1505. doi:10.1016/j.scitotenv.2017.07.054.

Karrman, A., Bavel,B., Jarnberg,U., Hardell,L. and Lindstrom, G. 2006. "Perfluorinated Chemicals in Relation to Other Persistent Organic Pollutants in Human Blood." *Chemosphere* 64: 1582–91. doi:10.1016/j.chemosphere.2005.11.040.

Kim, K.-H., Jahan, S. A., Kabir,E. and Brown, R. J. C. 2013. "A Review of Airborne Polycyclic Aromatic Hydrocarbons (PAHs) and Their Human Health Effects." *Environment International* 60. Elsevier Ltd: 71–80. doi:10.1016/j.envint.2013.07.019.

Kumar, S. N., Verma, P., Bastia,B. and Jain,A.K. 2014. "Health Risk Assessment of Polycyclic Aromatic Hydrocarbons: A Review." *Journal of Pathology and Toxicology* 1 (May 2016): 16–30.

Kuo, C. Y., Hsu, Y. W. and Lee, H. S.2003. "Study of Human Exposure to Particulate PAHs Using Personal Air Samplers." *Archives of Environmental Contamination and Toxicology* 44 (4): 454–59. doi:10.1007/s00244-002-1177-4.

Lai, I, Lee,C., Zeng,K. and Huang, H. 2011. "Seasonal Variation of Atmospheric Polycyclic Aromatic Hydrocarbons along the Kaohsiung Coast." *Journal of Environmental Management* 92 (8). Elsevier Ltd: 2029–37. doi:10.1016/j.jenvman.2011.03.026.

Machado, J., Chatkin, J. M., Zimmer, A. R., Goulart A. P., and Thiesen, F. 2014. "Cotinine and Polycyclic Aromatic Hydrocarbons Levels in the Amniotic Fluid and Fetal Cord at Birth and in the Urine from Pregnant Smokers." *PLoS ONE* 9 (12): 1–12. doi:10.1371/journal.pone.0116293.

Madhavan, N. D. and Naidu, K. A. 1995. "Polycyclic Aromatic Hydrocarbons in Placenta , Maternal Blood , Umbilical Cord." *Human & Experimental Toxicology*, no. 14: 503–6. doi:10.1177/096032719501400607.

Massey, J. B., and Pownall, H. J. 1998. "Surface Properties of Native Human Plasma Lipoproteins and Lipoprotein Models." *Biophysical Journal* 74 (2). Elsevier: 869–78. doi:10.1016/S0006-3495(98)74010-X.

Myllynen, P., Pasanen M., and Pelkonen, O. 2005. "Human Placenta : A Human Organ for Developmental Toxicology Research and Biomonitoring." *Placenta*, no. 26: 361–71. doi:10.1016/j.placenta.2004.09.006.

Oliva, M., González de Canales, M. L., Gravato, C., Guilhermino, L., and Perales, J. A. 2010. "Biochemical Effects and Polycyclic Aromatic Hydrocarbons (PAHs) in Senegal Sole (Solea Senegalensis) from a Huelva Estuary (SW Spain)." *Ecotoxicology and Environmental Safety* 73 (8): 1842–51. doi:10.1016/j.ecoenv.2010.08.035.

Pleil, J. D., Stiegel, M. A., Sobus J. R., Tabucchi, S., Ghio, A. J. and Madden, M. C. 2010. "Cumulative Exposure Assessment for Trace-Level Polycyclic Aromatic Hydrocarbons (PAHs) Using Human Blood and Plasma Analysis." *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* 878 (21): 1753–60. doi:10.1016/j.jchromb.2010.04.035.

Radmacher, P. G., Looney, S. W. and Myers, S. R. 2010. "Maternal and Cord Blood Plasma Polycyclic Aromatic Hydrocarbons in Maternal and Cord Blood Plasma." *Polycyclic Aromatic Compounds*, no. January 2015: 37–41. doi:10.1080/10406631003800639.

Ravindra, K., Sokhi, R., and Van Grieken, R. 2008. "Atmospheric Polycyclic Aromatic Hydrocarbons: Source Attribution, Emission Factors and Regulation." *Atmospheric Environment* 42 (13): 2895–2921. doi:10.1016/j.atmosenv.2007.12.010.

Ren, A., Qiu, X., Jin, L., Ma, J., Li, Z., Zhang, L., Zhu, H., and Finnell, R. H. 2011. "Association of Selected Persistent Organic Pollutants in the Placenta with the Risk of Neural Tube Defects." *Environmental Sciences* 108 (31): 12770–75. doi:10.1073/pnas.1105209108.

Sexton, K., Salinas, J. J., McDonald, T.J., Gowen, R.M. Z., Miller, R. P., McCormick, J.B., and Fisher-Hoch, S.P.. 2011. "Polycyclic Aromatic Hydrocarbons in Maternal and Umbilical Cord Blood from Pregnant Hispanic Women Living in Brownsville, Texas." *International Journal of Environmental Research and Public Health* 8 (8): 3365–79. doi:10.3390/ijerph8083365.

Shrivastava, A. and Gupta V. 2011. "Methods for the Determination of Limit of Detection and Limit of Quantitation of the Analytical Methods." *Chronicles of Young Scientists* 2 (1): 21. doi:10.4103/2229-5186.79345.

Stronkhorst, J., Ysebaert, T. J., Smedes, F., Meininger, P. L., Dirksen, S., and Boudewijn, T. J. 1993. "Contaminants in Eggs of Some Waterbird Species from the Scheldt Estuary, SW Netherlands." *Marine Pollution Bulletin* 26 (10): 572–78. doi:10.1016/0025-326X(93)90409-D.

Suk, W. A., Collman, G., and Damstra, T. 2014. "Human Biomonitoring: Research Goals and Needs." *Environmental Health Perspectives* 104: 479–83.

Tang, D., Li, T., Chow, J., Kulkarni, S., Watson, J., Ho,S., Quan, Z., Qu, L. and Perera, F. 2014. "Air Pollution Effects on Fetal and Child Development : A Cohort Comparison in China." *Environmental Pollution* 185: 90–96. doi:10.1016/j.envpol.2013.10.019.

Urbancova, K., Lankova,D., Rossner,P., Rossnerova,A., Svecova,V., Tomaniova,M., Veleminsky,M., Sram, R.J., Hajslova,J., and Pulkrabova,J. 2017. "Evaluation of 11 Polycyclic Aromatic

Hydrocarbon Metabolites in Urine of Czech Mothers and Newborns." *Science of the Total Environment* 577: 212–19. doi:10.1016/j.scitotenv.2016.10.165.

Wang, Z., Ren, P., Sun, Y., Ma, X., Liu, X., Na, G., and Yao, Z. 2013. "Gas/particle Partitioning of Polycyclic Aromatic Hydrocarbons in Coastal Atmosphere of the North Yellow Sea, China." *Environmental Science and Pollution Research* 20 (8): 5753–63. doi:10.1007/s11356-013-1588-y.

Yin, S., Tang, M., Chen, F., Li, T. and Liu, W. 2016. "Environmental Exposure to Polycyclic Aromatic Hydrocarbons (PAHs): The Correlation with and Impact on Reproductive Hormones in Umbilical Cord Serum." *Environmental Pollution*, 1–9. doi:10.1016/j.envpol.2016.10.090.

Yu, Y., Wang X., Wang, B., Tao S., Liu W., Wang X., Cao, J., Li B., Lu, X., and Wong, M.H. 2011. "Polycyclic Aromatic Hydrocarbon Residues in Human Milk, Placenta, and Umbilical Cord Blood in Beijing, China." *Environmental Science & Technology* 45 (23): 10235–42. doi:10.1021/es202827g.

Yuan, Y., Jin, L., Wang, L., Li, Z., Zhang, L., and Zhu, H. 2013. "Levels of PAH – DNA Adducts in Placental Tissue and the Risk of Fetal Neural Tube Defects in a Chinese Population" 37: 70–75.

Zhang, S., Zhang, W. and Wang, K. 2009. "Concentration , Distribution and Source Apportionment of Atmospheric Polycyclic Aromatic Hydrocarbons in the Southeast Suburb of Beijing , China." *Environmental Monitoring and Assessment*, 197–207. doi:10.1007/s10661-008-0261-2.

Zhang, X., Li, X., Jing, Y., Fang, X., Zhang, X., Lei, B. and Yu, Y. 2017. "Transplacental Transfer of Polycyclic Aromatic Hydrocarbons in Paired Samples of Maternal Serum, Umbilical Cord Serum, and Placenta in Shanghai, China." *Environmental Pollution* 222. Elsevier Ltd: 267–75. doi:10.1016/j.envpol.2016.12.046.

Zhang, Y. and Tao, S. 2009. "Global Atmospheric Emission Inventory of Polycyclic Aromatic Hydrocarbons (PAHs) for 2004." *Atmospheric Environment* 43 (4). Elsevier Ltd: 812–19. doi:10.1016/j.atmosenv.2008.10.050.

2.7 Supplementary Data

Table 2-S1 - Levels of naphthalene, phenanthrene, pyrene and benzo[a]pyrene equivalents (expressed in µg/mg protein) in placenta (homogenate fraction). Values represent mean and standard deviation (between brackets) of PAHs according to the area residence related characteristics reported in the questionnaires by parturient. N-number of replicates.

	Naphthalene	**Phenanthrene**	**Pyrene**	**Benzo[a] Pyrene**	**N** (%)	**N**
HABITATION						
Place of residence						
Urban	7.87 (3.32)	0.28 (0.14)	0.02 (0.01) *	$2.89x10^{-03}$ (0.003)	40.8%	20
Rural	8.50 (3.47)	0.30 (0.14)	0.04 (0.03)	$2.72x10^{-03}$ (0.0006)	34.7%	17
Intermediate	7.88 (3.16)	0.29 (0.11)	0.01(0.0004)	$3.76x10^{-03}$ (0.004)	24.5%	12
House near industrial activities						
Yes	7.39 (3.37)	0.26 (0.13)	0.03 (0.02)	$3.96x10^{-03}$ (0.004)	30.6%	15
No	8.31 (3.20)	0.30 (0.12)	0.01 (0.003)	$3.04x10^{-03}$ (0.003)	69.4%	34
House description						
Detached villa detached from other houses	6.60 (1.95)	0.23 (0.08)	<LOD	<LOD	10.2%	5
Detached villa next to one or more houses	7.97 (3.45)	0.29 (0.13)	0.03 (0.03)	$2.40x10^{-03}$ (0.002)	46.9%	23
Apartment	8.57 (3.40)	0.71 (1.79)	0.02 (0.009)	$3.26x10^{-03}$ (0.004)	38.8%	19
Other	7.19 (1.84)	0.24(0.07)	<LOD	$5.78x10^{-03}$ (0.004)	4.1%	2
Frequency of cars near house						
Constantly	9.05 (4.28)	0.33 (0.18)	0.02 (0.0084)	$4.18x10^{-03}$ (0.004)	26.5%	13
Frequently	8.22 (3.57)	0.29 (0.13)	<LOD	$1.23x10^{-03}$ (0.0006)	24.5%	12
Less Frequently	8.43 (2.10)	0.30 (0.08) *	0.01 (0.0004)	$1.82x10^{-03}$ ($1.18x10^{-03}$)	28.6%	14

Almost Nothing	5.92 (1.73)	0.21 (0.06)	0.03 (0.02)	$6.17x10^{-03}$ ($2.58x10^{-03}$)	20.4%	10
Frequency of heavy road vehicles near house						
Constantly	11.66 (6.14)	0.43 (0.24)	<LOD	<LOD *	6.1%	3
Frequently	9.36 (3.07)	0.34 (0.12)	<LOD	$3.56x10^{-03}$ (0.003)	14.3%	7
Less Frequently	8.86 (3.10)	0.32 (0.12)	0.01(0.0004)	$8.92x10^{-04}$ ($5.08x10^{-04}$)	28.6%	14
Almost Nothing	6.76 (2.47)	0.24 (0.10)	0.03 (0.02)	$4x10^{-03}$ (0.003)	51.0%	25

Table 2-S2 - Spearman correlations between levels of naphthalene, phenanthrene, pyrene and BaP equivalents in biological matrices and cotinine levels in mothers' plasma.

PAHs	Tissues	r	P value	N
Naphthalene	**Homogenized placenta**	-0.0751	0.607	49
	Umbilical cord cells	-0.0286	0.916	14
Phenanthrene	**Homogenized placenta**	-0.0591	0.685	49
	Supernatant placenta	0.829	0.05	6
	Umbilical cord cells	-0.2333	0.520	9
	Umbilical cord plasma	0.0115	0.948	32
	Mother plasma	-0.464	0.00486	36
Pyrene	**Homogenized placenta**	-0.236	0.490	10
	Supernatant placenta	-0.333	0.387	8
	Umbilical cord plasma	-0.167	(0.643	9
	Mother plasma	-0.165	0.949	13
Benzo[a]Pyrene	**Homogenized placenta**	0.0220	0.935	13
	Supernatant placenta	0.200	0.783	5
	Umbilical cord plasma	0.0549	0.849	13
	Mother plasma	-0.385	0.186	13

Table 2-S3 - Levels of naphthalene, phenanthrene, pyrene and benzo[a]pyrene equivalents (expressed in µg/mg protein) in placenta (homogenate fraction). Values represent mean and standard deviation (between brackets) of PAHs according to smoking habits and/or tobacco smoke exposure during pregnancy reported in the questionnaires by parturient. N-number of replicates.

	Naphthalene	**Phenanthrene**	**Pyrene**	**Benzo[a] Pyrene**	**N** (%)	**N**
Passive Smoker (Restaurants; coffee shops)						
Never	8.17 (3.31)	0.29 (0.13)	0.02 (0.02)	$3.25x10^{-03}$ (0.003)	53.1%	26
Sporadically	6.77 (2.39)	0.24 (0.10)	<LOD	<LOD	34.7%	17
Daily (1hour)	<LOD	<LOD	<LOD	<LOD	6.1%	3
Daily (+3hours)	<LOD	<LOD	<LOD	<LOD	6.1%	3
House						
Never	7.86 (2.99)	0.28 (0.12)*	0.02 (0.02)	$3.04x10^{-03}$ (0.003)	77.6%	38
Sporadically	7.83 (2.65)	0.30 (0.11)	<LOD	<LOD	6.1%	3
Daily (±1hour)	6.26 (1.16)	0.21 (0.05)	<LOD	<LOD	8.2%	4
Daily (+3hours)	11.52 (5.59)	0.42 (0.22)	<LOD	$1.30x10^{-03}$ ($6.79x10^{-05}$)	8.2%	3
Work Place						
Never	8.78 (2.79)	0.31 (0.11)	0.02 (0.01)	$3.97x10^{-03}$ (0.004)	91.8%	45
Sporadically	6.83 (2.90)	0.24 (0.12)	0.03 (0.02)	$3.21x10^{-03}$ (0.003)	4.1%	2
Daily (1hour)	5.75 (2.01)	0.20 (0.07)	<LOD	<LOD	2.0%	1
Daily (+3hours)	10.64 (6.85)	0.38 (0.27)	<LOD	$2.48x10^{-03}$ (0.001)	2.0%	1

Table 2-S4 - Levels of naphthalene, phenanthrene, pyrene and benzo[a]pyrene equivalents (expressed in µg/mg protein) in placenta (homogenate fraction). Values represent mean and standard deviation (between brackets) of PAHs according to work place related characteristics reported in the questionnaires by parturient. N-number of replicates.

	Naphthalene	**Phenanthrene**	**Pyrene**	**Benzo(a) Pyrene**	**N** (%)	**N**
WORK PLACE						
Occupation						
Unemployed	8.62 (2.67)	0.89 (1.70)	<LOD	$3.99x10^{-03}$ (0.003)	38.8%	19
Employed	7.76 (3.70)	0.28 (0.14)	0.02 (0.02)	$2.70x10^{-03}$ (0.002)	55.1%	27
Student	6.74 (1.82)	0.21 (0.14)	<LOD	<LOD	6.1%	3
Where is the occupation						
Aveiro	7.76 (3.18)	0.27 (0.12)	0.03 (0.02)	$3.45x10^{-03}$	81.6%	40
Outside Aveiro	8.51 (1.52)	0.31 (0.04)	0.02 (0.01)	<LOD	8.2%	4
Other	9.75 (5.30)	0.36 (0.20)	0.01 (0.002)	<LOD	10.2%	5
What was your last occupation						
Student	8.50 (3.43)	0.31 (0.12)	0.03 (0.02)	$2.44x10^{-03}$ (0.003)	40.4%	19
Work in same area	7.16 (3.88)	0.25 (0.15)	<LOD	<LOD	10.6%	5
Commerce work	7.47 (3.82)	0.26 (0.16)	0.02 (0.01)	$2.00x10^{-3}$ (0.001)	23.4%	11
Other	7.93 (2.51)	0.28 (0.10)	<LOD	$5.78x10^{-3}$ (0.004)	25.5%	12
Exposure to chemicals in workplace						
Yes	7.79 (2.61)	0.28 (0.10)	0.03 (0.02)	$4.39x10^{-3}$ (0.02)	46.9%	23
No	8.25 (3.68)	0.29 (0.14)	0.03 (0.004)	$2.15x10^{-3}$ (0.003)	53.1%	26

Chapter III

3.1 Final Remarks

Epidemiological studies are multidisciplinary studies that rely on different areas of research, from chemistry to biology and medical disciplines that perform human monitoring in order to understand patterns, causes, and effects on human health and disease development. The use of invasive and/or non-invasive matrices to assess human exposure to environmental contaminants is of upmost relevance. For the first time, a cross-sectional study concerning pre-natal exposure to PAHs was performed in Aveiro district, Portugal, an urban region with 78455 habitants, according to INE (Instituto Nacional de Estatística) (2001) and also with highly industrialized areas.

Aveiro, Ílhavo, and Albergaria-a-Velha were the counties of parturient residence presenting the highest levels of PAHs in placenta, while those from Águeda presented the lowest PAHs levels. Furthermore, higher LPAHs levels in placenta were associated with parturient residence near traffic roads (higher traffic exhaust). Aveiro and Ílhavo have in fact higher urban and industrial areas where PAHs emissions are more prone to occur. However, to better depict the main drivers accounting for the differences in PAHs levels among the different counties a higher number of mothers/newborn pairs from each county should be studied in the future. A higher and representative number of parturient from each municipality (e.g. in the present study N=3 from Albergaria-a-Velha; N=5 Estarreja) will help to understand and determine the main PAHs sources/emissions for each municipality.

In this research study, one of the main goals was to examine the influence of variables accounting for the levels of naphthalene, phenanthrene, pyrene and BaP equivalents in the biological matrices analyzed. Namely, tobacco exposure at work and traffic exhaust accounted for 15.2% of the variability observed in PAHs levels. There are other environmental and lifestyle variables that can account for the PAHs levels observed in parturient placenta and blood, namely mothers' eating habits. PAHs may occur in food preparation (e.g. grilled food), conservation and storage. PAHs levels were found in some products such as dried fruits, olive pomace oil, smoked fish, grape seed oil, smoked meat products, fresh mollusks, spices/sauces and condiments (Buckpitt *et al*., 2002; Yu *et al*., 2011). Therefore, in future studies the relation of PAHs levels present in placenta, umbilical cord blood and mothers' blood and parturient eating habits should be explored.

In the last decade, Portugal has been one of the most affected European countries with forest fires (JRC, 2015), with 2017 being considered the worst year in the last decade. It is expected that due to climatic changes, these natural catastrophes will even tend to increase exposure in human populations to high concentrations of PAHs through air. In this context, it would be relevant to perform regular human biomonitorization studies in critical burned areas of Portugal in order to understand if forest fires are contributing to PAHs levels in placenta, umbilical cord blood and parturient' blood.

There are several studies that use PAHs levels in placenta and blood as biomarkers of exposure in pregnant women and their newborns (Al-Saleh *et al*., 2013; Machado *et al*., 2014; Chen *et al*., 2014; Zhang *et al*., 2017). Blood and placenta are good matrices for PAHs levels measurements. Placenta is a large non-invasive matrix that can trace possible PAHs partial bioaccumulation because of their lipophilic properties and can provide possible information about the mothers' exposure to PAHs levels. In this research the highest PAHs levels were found in placenta. Placenta had higher PAHs levels than umbilical cord and mothers' blood. Umbilical cord and parturient blood can supply significant information about mothers' and fetus exposure. Since these matrices allow us to obtain information about the PAHs levels in the human organism, an indicative and/threshold levels would be of extreme relevance to develop or adapt for placenta and blood matrices in order to assess the risk of exposure of the studied populations based on the obtained PAHs levels.

However, the use of different methodologies to quantify PAHs turns difficult the comparison of pre-natal exposure among the different populations studied. The 1OH-Pyrene, metabolite of pyrene quantified in urine, is the most used biomarker of exposure to PAHs. It is commonly used to assess total burden of PAHs in occupational groups; a level of about 0.5 μmol 1OH-Pyrene /mol creatinine in urine is considered indicative of occupational exposure to PAHs by the American Conference of Governmental Industrial Hygienists (ACGIH, 2010). The quantification of PAHs metabolites in urine might be a helpful non-invasive biomarker of exposure to characterize parturient exposure to PAHs and to complement data obtained in placenta and blood matrices.

Considering the different methodologies commonly used for PAHs quantification in human biological matrices, the establishment of a standard methodology for PAHs measurement is pertinent and will allow for comparisons between populations of different countries and under different environmental and

anthropogenic pressures. In the present study, the PAHs quantifications were performed using fixed fluorescence wavelength methodology. However, there are other PAHs methodologies like HPLC and GC-MS. In future studies, it would be interesting to compare the PAHs measurements performed through the different techniques.

Biomarkers of exposure have shown to be essential tools to study the relationship between environmental exposure and health and disease development. Besides in the present study no significant correlations were found between PAHs levels in placenta and anthropometry data of newborns, in general, higher levels of PAHs were found in groups of newborns with less weight, height and cephalic perimeter. Taking these results into account, further longitudinal or cross-sectional studies should be conducted regarding intrauterine exposure to PAHs, which may affect the fetal development, in order to prevent negative outcomes in Portuguese populations and offspring.

3.2 References

ACGIH, 2010. Documentation for a Recommended BEI of Polycyclic Aromatic Hydrocarbons. American Conference of Governmental Industrial Hygienists, Cincinatti, Ohio, USA.

Al-Saleh, I., Alsabbahen, A., Shinwari, N., Billedo, G., Mashhour, A., Al-Sarraj, Y., El Din Mohamed, G., and Rabbah, A. 2013. "Polycyclic Aromatic Hydrocarbons (PAHs) as Determinants of Various Anthropometric Measures of Birth Outcome." *Science of the Total Environment* 444. Elsevier B.V.: 565–78. doi:10.1016/j.scitotenv.2012.12.021.

Buckpitt, A., Boland, B., Isbell, M., Morin, D., Shultz, M., Fanucchi, M., Van Winkle, L. and Plopper, C. 2002. "Naphthalene-induced respiratory tract toxicity : metabolic mechanisms of toxicity" 34 (4): 791–820. doi:10.1081/dmr-120015694.

Chen, Q., Zheng, T., Bassig, B., Cheng, Y., Leaderer, B., Lin, S., Holford, T., Qiu, J., Zhang, Y., Shi,K., Zhu, Y., Niu,J.,Li, Y., Guo, H., Hu, X., Jin, Y. 2014. "Prenatal Exposure to Polycyclic Aromatic Hydrocarbons and Birth Weight in China." *Open Journal of Air Pollution* 3 (December): 100–110. doi:10.4236/ojap.2014.34010.

INE (Instituto Nacional de Estatística). 2001. *CENSOS 2001*.

JRC, 2015. Forest Fires in Europe, Middle East and North Africa 2014. Joint Research Centre Technical Reports. Joint Report of JRC and Directorate-General Environment, Luxembourg.

Machado, J., Chatkin, J. M., Zimmer, A. R., Goulart A. P., and Thiesen, F. 2014. "Cotinine and Polycyclic Aromatic Hydrocarbons Levels in the Amniotic Fluid and Fetal Cord at Birth and in the Urine from Pregnant Smokers." *PLoS ONE* 9 (12): 1–12. doi:10.1371/journal.pone.0116293.

Yu, Y., Wang X., Wang, B., Tao S., Liu W., Wang X., Cao, J., Li B., Lu, X., and Wong, M.H. 2011. "Polycyclic Aromatic Hydrocarbon Residues in Human Milk, Placenta, and Umbilical Cord Blood in Beijing, China." *Environmental Science & Technology* 45 (23): 10235–42. doi:10.1021/es202827g.

Zhang, X., Li, X., Jing, Y., Fang, X., Zhang, X., Lei, B. and Yu, Y. 2017. "Transplacental Transfer of Polycyclic Aromatic Hydrocarbons in Paired Samples of Maternal Serum, Umbilical Cord Serum, and Placenta in Shanghai, China." *Environmental Pollution* 222. Elsevier Ltd: 267–75. doi:10.1016/j.envpol.2016.12.0466.